# An assessment of microplastics in two freshwater ecosystems in Gauteng

By

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## Cases

°C ..... Degrees Celsius

µ ..... Micro

µ-FT-IR ..... Micro-Fourier Transform-Infrared Spectroscopy

ADW ..... After Dam Wall

Al ..... Aluminium

ASTM ..... American Society for Testing and Material Standards

BFS ..... Braamfontein Spruit

Br ..... Bromine

C ..... Confluence

Cd ..... Cadmium

Cl ..... Chlorine

dw ..... Dry weight

DWAF ..... Department of Water and Sanitation

DDT ..... Dichlorodiphenyltrichloroethane

EDCs ..... Endocrine Disrupting Chemicals

Fe ..... Iron

G ..... Gabion

g ..... Gram

GESAMP ..... Group of Experts on the Scientific Aspects of Marine Environmental Protection

g $L^{-1}$ ..... Gram per litre

Hg..... Mercury

$H_2O$ .....Water

IBM SPSS .....International Business Machines: Statistical Package for the Social Sciences

kg .....Kilogram

km .....Kilometer

KOH .....Potassium hydroxide

L .....Litre

LVC .....Loch Vaal Club

m .....Meter

$m\ s^{-1}$ ..... Meter per second

$m^3.s^{-1}$ ..... Cubic meter per second

MERI .....Marine and Environmental Research Institute

min .....Minutes

mL ..... Mililitres

mm .....Milimetres

Mn ..... Manganese

NaCl .....Sodium Chloride

OCPs..... Organochloride Pesticides

Particles $g^{-1}$ ..... Particles per gram

Particles $g^{-1}$ fish.....Particles per gram of fish

Particles $g^{-1}$ gut ..... Particles per gram of gut

Particles $kg^{-1}$.....Particles per kilogram

Particles $m^{-3}$ .....Particles per cubic meter

Particles.$mg^{-1}$ .....Particles per miligram

Pb ..... Lead

PBDE ..... Polybrominated Diphenyl Ether

pH ..... Potential of hydrogen

ppm ..... Parts per million

QGIS ..... Quantam Geographic Information System

R ..... Rapids

RS ..... Raw sewage

RHAM ..... Rapid Habitat Assessment Methodology

rpm ..... Rotations per minute

RDA ..... Redundancy Analysis

SANS ..... South African National Standards

T ..... Tributary

TDS ..... Total Dissolved Solids

US ..... United States

USEPA ..... United States Environmental Protection Agency

VD ..... Vaal Dam

VR ..... Vaal Rus

Sed depth ..... Depth of water was recorded where sediment was collected

Sed velocity ..... Water velocity recorded where sediment was collected

sp ..... Species

W ..... Weir

ww ..... Wet weight

µg $g^{1}$ ..... Micrograms per gram

µm ..... Micrometers

µS.$cm^{-1}$ ..... Microsiemens per centimeter

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# SUMMARY

South Africa is a country rich in biodiversity, however, the need for growth in the country has to some extent outweighed the importance of its biodiversity. This has led to various ecological problems from acid mine drainage, loss of habitat, and the polluting of important freshwater bodies leading to the loss of biodiversity. One pollutant that has been described world-wide and has been discovered in various environments is called microplastics. Unfortunately, the extent of microplastic pollution in South African freshwater ecosystems is currently unknown.

Microplastics are defined as plastics smaller than 5 mm up to 0.05 mm in size. These plastics enter the environment and undergo certain physical changes, most notably density changes and an increase of surface area. These changes allow plastics to release harmful additives such as flame retardants. The changes furthermore allow plastics to absorb toxins from the surrounding environment, most notably metals like Pb, Cd and organochloride pesticides such as DDT. These plastics may then enter the food chain from producers to top predators in marine, freshwater, soil and terrestrial environments.

In this study, microplastics abundances in two freshwater ecosystems were investigated. The aim of these studies was to determine if there are microplastics in the two different freshwater ecosystems, but most importantly, the greater aim was to determine how microplastics behave and distribute in an ecosystem. Two microplastic profiles of a smaller stream in an urbanized setting (the Braamfontein Spruit) and a higher order river (the economically and ecologically important upper Vaal River) were constructed to achieve this aim.

Water, sediment and biota were investigated in the two water bodies. In the Braamfontein Spruit, the benthic macroinvertebrate *Chironomus* spp. were investigated for microplastics with sediment and water. Nine sites were selected along the stream and various stream characteristics, such as weirs, tributaries and rapids, were considered when the sites were selected. Sampling took place over one day to create a snapshot image of the microplastic distribution. Various stream characteristics such as water velocity, depth and sediment grain profiles, were taken at each site. Microplastics were detected in water (705 particles $m^{-3}$), *Chironomus* spp.

larvae (56.2 particles $g^{-1}$ ww) and sediment (166.8 particles $kg^{-1}$ dw). Microplastics showed a distinct distribution pattern as the microplastics distributed throughout the stream. The results indicated how increased water velocity and lower depth increases microplastics in water and similarly, a reduction in stream velocity and increased depth would increase microplastics in the sediment, which allowed an increase of microplastics in benthic macroinvertebrates. The sediment grain profiles similarly indicated that areas with a smaller sediment grain size were able to trap more microplastic particles.

The microplastic profile of the larger upper Vaal River was similarly created. Water, the benthic fish *Clarias gariepinus* and sediment were investigated along four sites. Sites were selected above and below the Vaal Dam wall and Vaal River Barrage weir, to determine how the obstruction would influence microplastic distribution. Microplastics were detected in all three matrices with a mean concentration of 3299.58 particles $m^{-3}$ in water, 7.47 particles per fish and 46.7 particles $kg^{-1}$ in sediment.

Microplastic distribution of the various sites showed a similar relationship to the results found in the smaller Braamfontein Spruit. In the Vaal Dam and Vaal River Barrage, the slower moving to still water showed low microplastic levels in the water but yet again an increased microplastic concentration was detected in the fast-flowing water. Below the Vaal Dam wall, where an average of 17.19 $m^{-3}$ of water is released per second a microplastic concentration of 12398.33 particles $m^{-3}$ was detected. Yet again the benthic organisms tended to have a similar distribution pattern to that of sediment. It was concluded that dams may act as sinks, trapping large quantities of microplastics in the sediment, before releasing it in high concentrations downstream.

The results in both study locations indicate the same conclusion. Microplastic are not evenly distributed in an ecosystem. They migrate both horizontally and vertically in the water column depending on the surrounding environment. The significance of the results is that in order to determine the influence of microplastics on organisms and to determine the levels of microplastics in a freshwater body, stream and niche characteristics must be considered. Similarly, the study recommends that biota, water and sediment must be investigated together and that simply investigating water in a series of dams along a greater riverine system would provide a false indication of total microplastic content in an ecosystem.

# CHAPTER 1
# INTRODUCTION

# Chapter 1

## 1.1 General introduction to microplastics

One of the most important resources for living organisms is fresh water (Chaplin, 2001). Freshwater is not only important for drinking, but is required for agriculture, basic hygiene and therefore the health of a large population (Chaplin, 2001). In South Africa the use and management of water plays an important role in the success of the country, however activities such as large scale mining, raw sewage entering rivers and the misuse of water has led to a scenario where water has become a dwindling resource in some parts of the country (Oberholster et al., 2008; Wepener et al., 2011; Weideman et al., 2019). One pollutant, however, has remained unseen in the South African freshwater environment. This pollutant is called microplastics (Arthur et al., 2009).

From the moment a person wakes up, to the moment they finally end their day, they are constantly connected to plastic. This is clearly indicated where up to 322 million tonnes of plastic had been produced by 2016 while it is estimated that by 2050 over 33 billion tonnes would be produced globally (Horton et al., 2016; Li et al., 2018). Very little thought goes into the plastics used daily, and where it will eventually end up. One possible outcome for these plastics, is being broken down into smaller particles, called microplastics (Li et al., 2018). Microplastics are plastic materials of between 5-0.05 mm in size and are further classified as being either primary microplastics, such as small beads, or secondary microplastics, from larger plastic products like microfibres or shards (Arthur et al., 2009; Hidalgo-Ruz et al., 2012; Blettler et al., 2018; Li et al., 2018). Once these microplastics enter the environment, certain morphological changes can occur (Li et al., 2018; Guo and Wang, 2019). Structural changes that occur in microplastics include discolouration, breaking down into smaller particles, density changes from microbial growth and most notably an increase of surface area due to the surface fracturing (Guo and Wang, 2019). These changes allow microplastics to release toxicants used in their production, some of which are Endocrine Disrupting Chemicals (EDCs) and allow them the ability to absorb high amounts of toxicants from the environment due to their enlarged surface area (Wang et al., 2017; Collicutt et al., 2019; Guo and Wang, 2019). This has been noted in

various studies where high concentrations of a variety of metals and Organochloride Pesticides (OCPs) have been detected on the surface of microplastics (Guo and Wang, 2019).

The environmental pathway of microplastics does not end with the gathering of toxins from the environment. As microplastics discolour, increase in density and spread in aquatic systems, they can form part of the food chain for aquatic and terrestrial species. Microplastics can enter the food chain by being consumed by filter-feeding zooplankton, as found in studies by Sun et al. (2019). These zooplankton may then be secondarily ingested by larger predators that feed on them. Some species of animals may feed on these plastics directly, such as planktivorous fish, which have been found to mistake microplastics for smaller invertebrates (Ory et al. 2017). These plastics can then be passed on to larger animals through the food chain and have been discovered in many larger aquatic organisms such as the finless porpoise (*Neophocaena asiaeorientalis sunameri)* by Xiong et al. (2018), Chinook salmon (*Oncorhynchus tshawytscha)* by Collicutt et al. (2019), Skipjack tuna (*Katsuwonus pelamis*) by Rochman et al. (2015) and great marine mammals such as Sperm whales (*Physeter macrocephalus*) in the Mediterranean sea by de Stephanis et al. (2013). These are only a few examples of how microplastics have been found to move through the trophic system to secondary and tertiary consumers (Digka et al., 2018). Microplastics have also recently been detected in humans and multiple food sources they consume including fish, honey, salt and beer (Peixoto et al., 2019; Rainieri and Barranco , 2019). These findings indicate that microplastics may pose a threat to human health, although further investigations are necessary.

Not only do microplastics have possible effects on human health, they also carry potential environmental risks. The microplastics ingested by aquatic organisms could lead to a host of negative effects from gastrointestinal blockages, reduced reproduction, oxidative stress, growth delays, inflammation, cancer and the death of the animal (Lei et al., 2018; Xiong et al., 2019; Gatidou et al., 2019; Herrera et al., 2019). It has been discovered that microplastics could contribute to coral bleaching, a reduction in the ability for phytoplankton to photosynthesise and possibly the inhibition of fish species expelling certain toxicants such as mercury from their systems(Barboza et al., 2018; Syakti et al., 2019; Wang et al., 2019). These issues relate to some of the greatest natural disasters currently taking place on Earth.

## 1.2 History of microplastic research

The body of research on microplastics has grown exponentially in the last decade (Blettler et al., 2018, Eerkes-Medrano and Thompson, 2018; He et al., 2019). However, the first discovery of microplastics can be traced back as far as the late 1970s where microbeads were discovered along the shoreline of New Zealand by Gregory et al. (1977). Similarly, discoveries had been made in the Mediterranean Sea and North-West Atlantic Ocean during the same period (Blettler et al., 2018; Eerkes-Medrano and Thompson, 2018; Li et al., 2018). The first modern plastic polymer (Polyethylene) was produced in the 1930s, which suggests that plastics may have been in the environment for 40 years before the first microplastics were observed and recorded (Turner, 2019). Today, microplastics have been discovered across the world in every ocean and on every continent, from the polar oceans to the equator and from the sea surface to the Marianas Trench and even within sea ice (Rochman et al. 2015; Horton et al., 2017; Blettler et al. 2018; Eerkes-Medrano and Thompson, 2018; Li et al. 2018; Peng et al. 2018; Geilfus et al., 2019).

With the increase in publications on microplastics in the environment over the past decade, a clear trend in research bias was detected in review studies (Figure 1) by both Blettler et al. (2018), Eerkes-Medrano and Thompson (2018) and He et al. (2019). The researchers discovered that microplastic research had a much greater focus on the marine environment than the freshwater environment. Eerkes-Medrano and Thompson (2018), discovered that in 2017 only 20 publications on microplastics had been from the freshwater environment, with over 100 publications on the marine environment that year (Figure 1A). When the total publications on microplastics in these two environments were recorded, from various scientific databases between 1980 to May 2018, it was discovered that only a mere 13% of all publications had been from freshwater environments (Blettler et al., 2018). A review article by Eerkes-Medrano and Thompson (2018), found similar results with fewer publications of microplastic in freshwater environments between 2016 to 2017 as seen in Figure 1C. The low research output on microplastics in freshwater environments could be due to the popularity of research in the marine environment or could be attributed to the difficulty of detecting microplastics in freshwater ecosystems. There has recently been some expansion in the different media investigated for microplastic research. This is

indicated in Figure 1B, where the author He et al. (2019) indicates the total number of publications in which soil was investigated for microplastics had increased rapidly over time and similarly, a publication by Rezaei et al. (2019) and review article by Akdogan and Guven (2019), depicted the need for microplastic research in the atmosphere.

Regarding biota that had been investigated for microplastic and plastic content, fish species had been investigated most predominantly compared to other animal groups (de Sá et al., 2018). The other most investigated group was Mollusca, with Amphibians, Porifera and Nematoda being the least investigated groups of animals in microplastic studies (de Sá et al., 2018). The low total research output of microplastics in freshwater ecosystems remains a major concern when freshwater sources become increasingly polluted and unavailable for human and animal consumption, particularly in the South African context.

With increased awareness of microplastics in the environment, more research has been conducted not only on microplastics in the freshwater environment but how it behaves and spreads, to better understand how microplastics might influence the ecosystem (Peng et al., 2018; Rezaei et al., 2019; Dahms et al., 2020; Kane et al., 2020; Weideman et al., 2020). Only through understanding its role in the environment, will we be able to truly understand its effects on the ecosystem. This study aims to assess the microplastic profiles of an urban stream and large river, to understand how microplastics would distribute in the freshwater environment.

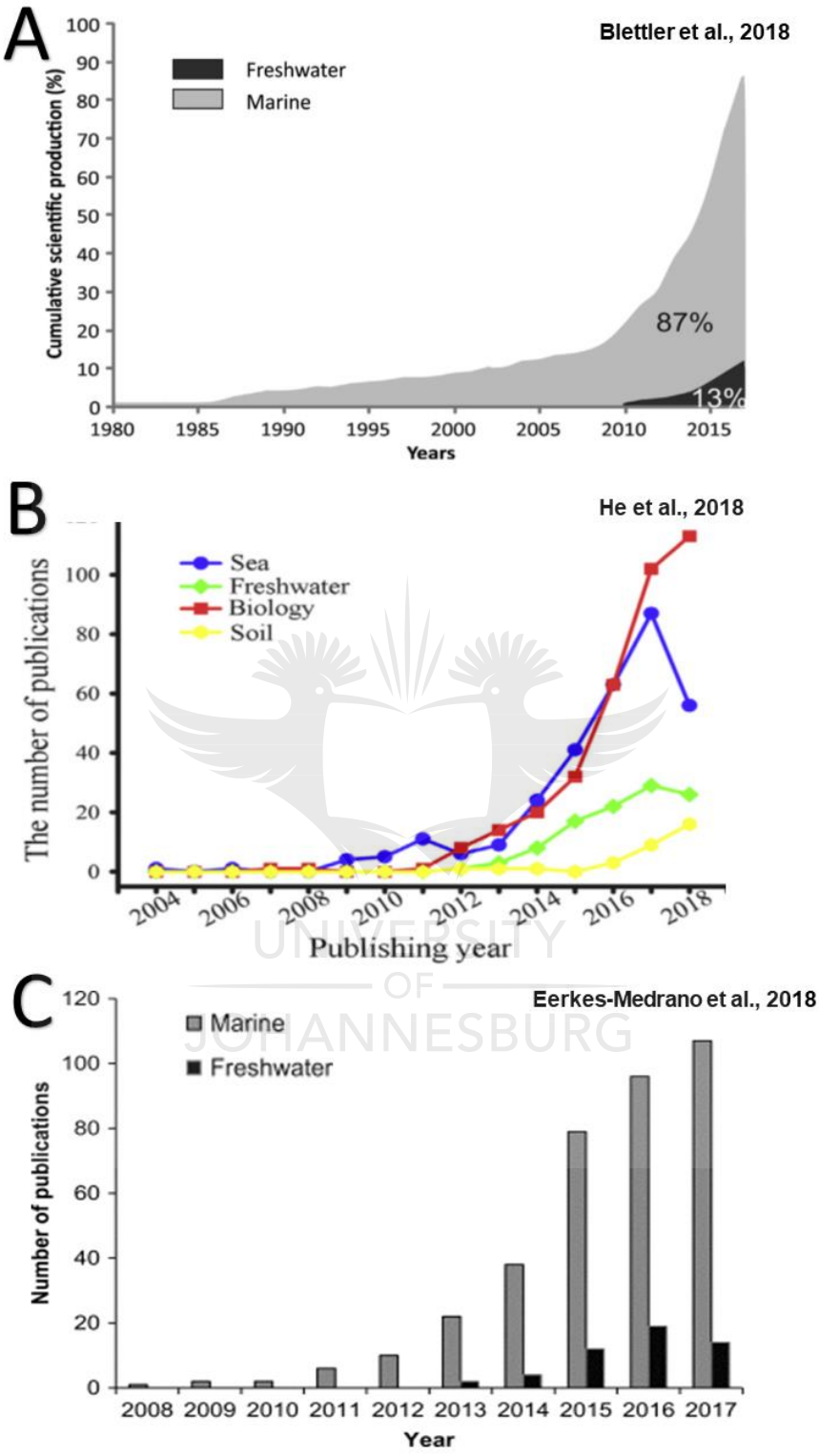


Figure 1: A combination of the results of three review articles on microplastics in the environment which depicts the areas that are lacking critical research based on the total number of publications per year and the environment that was investigated. A (Blettler et al., 2018), B (He et al., 2018), C (Eerkes-Medrano and Thompson, 2018).

## 1.3 Hypotheses, aims and objectives

### 1.3.1 Hypotheses

Due to the nature of the project, separate hypotheses were considered for the methodological review and primary research locations as described in Chapter 2.3 “Experimental design”.

#### 1.3.1.1 The Braamfontein Spruit

i. Microplastics will be detected in water, sediment and macroinvertebrates in the Braamfontein Spruit.
ii. Microplastic occurrence will be influenced by stream characteristics like stream velocity, depth and man-made structures.
iii. Benthic macroinvertebrates will show a similar microplastic accumulation trend as the sediment that they inhabit.

#### 1.3.1.2 Upper Vaal River

i. Microplastics will be found in the gastrointestinal tract of the benthic fish species *Clarias gariepinus* throughout the upper Vaal River system.
ii. A high percentage of the fish will contain secondary microplastics such as fibres.
iii. Large dams will be a collection point for large quantities of microplastics.
iv. Microplastic distribution will be influenced by environmental characteristics.



### 1.3.2 Aims

Several aims were set to establish the success of the project.

i. Validate methods through determining the microplastic profile of the Braamfontein Spruit.
ii. Establish the presence, or lack thereof, of microplastics (0.05-5mm) in the gastrointestinal tract of *Clarias gariepinus* in the upper Vaal River system.

iii. Determine the prevalence and abundance of microplastics throughout the upper Vaal River system in water, sediment and *Clarias gariepinus.*

iv. Determine the most prevalent class of microplastics in all three study components within the Braamfontein Spruit and upper Vaal River.

### 1.3.3 Objectives

Multiple objectives had to be accomplished for the success of the aims that were set.

i. Successfully adapt, develop and prepare methods to sample and analyse microplastics in water, sediment and biota in the South African context.

ii. Identify and classify microplastics collected in the study

iii. Statistically analyse and understand the relationship of microplastics in the Braamfontein Spruit and upper Vaal River.

## 1.4 Chapter outlines

Chapter 1: *Introduction*

This chapter aims to introduce the topic of microplastics and microplastic research in the environment, its shortcomings and what the study aims to achieve. It also highlights the hypotheses, aims of the study and the objectives set to determine its success. It then briefly outlines the scope of each chapter.

Chapter 2: *Literature review*

This chapter will primarily focus on the background information on microplastic research in South Africa. It will discuss the two primary locations used in this study and reasoning for their selection, with photographs of the sampling points that were investigated in the various chapters. The chapter then concludes with a discussion on the experimental design for the study and conclusion.

Chapter 3: *The microplastic profile of the Braamfontein Spruit*

This chapter investigates the microplastic abundances in water, sediment and the benthic macroinvertebrate *Chironomus* spp., as well as stream characteristics in the

Braamfontein Spruit, Johannesburg. It then discusses how the various stream characteristics might influence the spread of microplastics the aquatic environment. It concludes with the ending remarks and recommendations for future microplastic research. This chapter has already been published in the peer reviewed journal Science of the Total Environment “The microplastics profile of an urban African stream” (Dahms et al., 2020). The chapter does not consist of the published version of the manuscript as various sections such as the materials and methods, results and discussion had been expanded for the use in the dissertation. The first page of published manuscript is included in Annexure 4 as well as the authors’ statement in Annexure 3.

**Author contributions:**

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Writing- original draft, Formal analysis, Visualization, Investigation,

**G.J. van Rensburg**

Formal analysis, Writing- review and editing, Visualization, Conceptualization.

**R. Greenfield**

Supervision, Writing- review and editing, Funding acquisition.



Chapter 4: *Microplastics in the upper Vaal River*

This chapter consists of the analysis of microplastics in the upper Vaal River. Microplastics were analysed in water, sediment and in the gut of the benthic fish species, *Clarias gariepinus* to determine the microplastic abundances of the river. A comprehensive discussion of the microplastic profile in the river follows and how the construction of large dams may influence microplastic abundances. It concludes with the primary insights and what future microplastic studies in South Africa might be required.

Chapter 5: *Discussion*

This chapter uses the results in the study to discuss the role of microplastics in the environment adding aspects from both the terrestrial environment and atmosphere with urban streams and large “hard-working” rivers. It discusses the dangers of

microplastics and the role the environment plays in its spread and transport from both abiotic and biotic factors. It concludes by highlighting the importance of microplastic studies in South Africa.

Chapter 6: *Conclusion*

This chapter consists of the final concluding remarks of the study with recommendations for future microplastic research.

Chapter 7*: References*

This chapter consists of the literature cited in the study.

# Chapter 2
# Literature review

# Chapter 2

## 2.1 Microplastic research in South Africa

Microplastic research, not only in South Africa but the whole of Africa, is lagging behind other countries, with the full extent of microplastics in Africa not completely understood or represented. Microplastics have been detected in Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) within Lake Victoria, in freshwater snails (*Lanisters varicus* and *Melanoide tuberculate*) in the Osun River, on the beaches of Lake Malawi and reservoirs around Africa (Biginagwa et al., 2016; Akindele et al., 2019; Dalu et al., 2019; Mayoma et al., 2019). This clearly outlines the further need for microplastic research in Africa with so many predominant waterbodies in Africa containing microplastics.

In South Africa, microplastics have been detected along coastal areas, beaches and in some marine fish species such as the estuarine mullets (*Mugil cephalus*) with few studies being conducted on the freshwater sources in the country (Naidoo et al., 2015; Nel and Froneman, 2015; Verster et al., 2017). Recently, more research has been conducted in South Africa's scarce freshwater resources and microplastics have been detected in the water contained in dams within the Orange-Vaal River system, within sediment and macroinvertebrates in the Bloukrans River system in the Eastern Cape Province and more recently in water, sediment and macroinvertebrates in the Braamfontein Spruit in Johannesburg, South Africa's largest city (Nel et al., 2018; Weideman et al., 2019; Dahms et al., 2020). These rivers and streams all form part of major river basins within the country and it is therefore of utmost importance that microplastic research in freshwater sources in South Africa should continue.

Microplastic investigations such as those by Nel et al. (2018), Dahms et al. (2020) as well as Dikareva and Simon (2019), where several aspects of a stream or river are investigated may provide a more accurate base for exposure studies of microplastics in biota, and thus determine the full effect of microplastics in the ecosystem. It is critical to investigate multiple aspects of an ecosystem including the sediment, water column and biota, in order to provide a more holistic view of microplastics in the ecosystem.

## 2.2 Study sites

### 2.2.1 The Braamfontein Spruit (Location A)

The Braamfontein Spruit (map in Figure 4) flows through multiple suburbs and urban green zones in Johannesburg, South Africa (City parks, 2014). The stream has two major tributaries, the Westdene and Montgomery Spruits, which join the stream as it passes through several suburbs (City parks, 2014). The stream then flows into the larger Jukskei River, which is a tributary of the Limpopo River, a transboundary river and important resource to several African countries such as Botswana, Zimbabwe and Mozambique (City parks, 2014). At location A, a total of nine study points (site photos in Figure 2 and map of study area in Figure 4), were investigated in one day to establish a snapshot of how the various stream characteristics would influence microplastic abundances and to validate the methods used in the studies.

*Sampling point 1*

Point 1 was the uppermost section of the Braamfontein Spruit used in this study. It was located within the suburb Parkhurst approximately 1.4 km North East of the Emmarentia Dam in Johannesburg. The section of stream was located in a highly urbanised area, only a few hundred meters from a busy road at the time of the study. The water was observed to be foamy, with little evidence of plastic pollution. The stream had strong flow where microplastic levels would be expected to be relatively low here compared to the points downstream.

*Sampling point 2*

Point 2, the Montgomery Spruit, is a tributary to the Braamfontein Spruit. It is located not more than 20 m from point 1 before the confluence with the Braamfontein Spruit. The section of stream was more polluted with larger macroplastics than point 1 as seen in the riparian vegetation where large amounts of larger macroplastics were trapped. The flow of the stream seemed slow compared to that of the Braamfontein Spruit.

*Sampling point 3*

Point 3 is the confluence of the Braamfontein Spruit (point 1) and its tributary the Montgomery Spruit (point 2) and was located approximately 30 m downstream from

the first sampling points. Here, large and small plastics were observed in the stream with foam passing down the stream.

*Sampling point 4*

Point 4 was found approximately 1.9 km downstream from point 3 next to Delta Park in the suburb Craighall Park in Johannesburg. A few meters below this point a large weir was located which altered the structure of the stream. Flow was decreased compared to point 3, but a steady flow of surface water moved over the weir and the width of the stream increased upstream of the weir. Little to no macroplastics were observed in the water or surrounding vegetation and the water appeared to be clear.

*Sampling point 5*

Point 5 was only 50 m downstream from point 4 but below the large weir. The weir allowed for consistent flow with decreased depth and more rapids downstream. There was more macroplastic litter observed compared to the weir at point 4. The rapid change of the characteristics of the stream due to the weir may affect microplastic abundances above and below the weir.

*Sampling point 6*

Point 6 was located 3.1 km downstream of point 5 in the suburb Glenadrienne. The stream at this point was surrounded by parks with only a few residential buildings on the one bank. The topography of the area changed here with the stream forming more rapids with increased flow due to steep drop in height downstream. An increase of plastic litter was clearly observed in the vegetation on the bank of the stream. Microplastics loads were expected to increase, however the large rocky terrain may lead to fewer microplastics in the sediment.

*Sampling point 7*

Point 7 was located 4.7 km downstream of point 6 in the suburb Duxberry. The stream at this point, has passed through multiple highly urbanised areas with the impact visible as the water was observed to be more polluted with macroplastics found in high quantities. The sampling point was selected due to the presence of a large gabion, a manmade obstruction in the stream, which could influence microplastic abundance similarly to the weir at sampling points 4 and 5. Water was able to slowly pass through the gabion, but a similar effect was created compared to the weir between sampling

points 4 and 5. Flow decreased dramatically, which may again influence microplastic loads in the stream.

*Sampling point 8*

Point 8 was a further 2.8 km downstream of point 7 in a gated community in Rivonia. At this sampling point, the first clear signs of sewage entering the system were observed. The stream had few obstructions visible and streamflow rapidly increased compared to the previous point. Few macroplastics were seen on the bank of the river, possibly due to clean up initiatives from citizens within the gated community, as highlighted by a resident at the sampling point on the day of sampling. Microplastic levels were expected to be highest in the stream here from the increased flow and raw sewage.

*Sampling point 9*

Sampling point 9 is a further 2.6 km downstream of point 8 within Paulshof. By this point the stream had flowed underneath a freeway, past dense vegetation and through more urbanized areas than the previous green urban zones. At this sampling point the streamflow was observed to have slightly decreased compared to point 8. The riverbanks again show signs of macroplastics, however the sewage runoff seen in point 8 had somewhat dispersed.

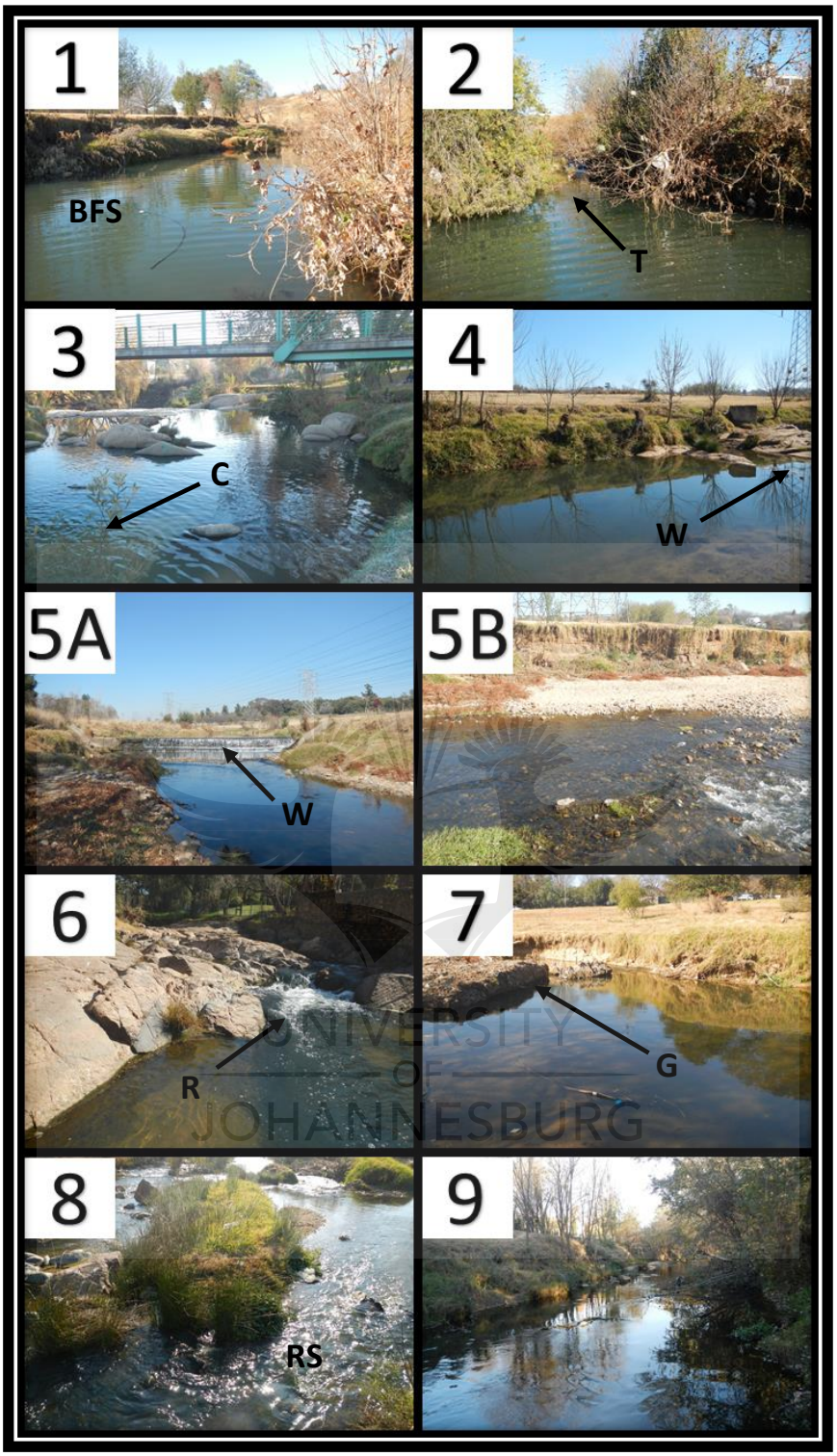


Figure 2: Photos of the Braamfontein Spruit where sampling took place. Sampling points move downstream from 1 to 9. 1- Braamfontein Spruit (BFS), 2- tributary the Montgomery Spruit (T), 3- confluence (C), 4- above weir (W), 5- below weir (5A indicating weir and B the sampling point), 6- rapids (R), 7- gabion (G), 8- rapids with possible raw sewage (RS), 9- final sampling point before confluence with the Jukskei River

## 2.2.2 The upper Vaal River (Location B)

The Vaal River forms part of the Orange-Vaal River system, the largest river system in South Africa (Weideman et al., 2020). The Vaal River itself makes up 1300 km of the system (Wepener et al., 2011). It has been described as one of the hardest working rivers in Africa with an unmeasurable value to South Africa (Wepener et al., 2011; Weideman et al., 2019). Gauteng, which is the most populated and economically important province of South Africa, relies heavily on the Vaal River to provide water to the people and industries in the area. The 63 km stretch of the Vaal River from the Vaal Dam to the Vaal River Barrage (map in Figure 11) remains important, as run-off from three metropolitan areas, gold mines and industries flow into the river between these sites (Wepener et al., 2011). This stretch of river has been described as a highly polluted waterbody within South Africa (Wepener et al., 2011; Weideman et al., 2019). A total of 4 sampling points (Figure 3) were investigated in the upper Vaal River. To determine more accurate microplastic levels, sampling took place before and shortly after the prominent Vaal Dam wall and Vaal River Barrage weir (Figure 18C and D) of the Vaal Dam and Vaal River Barrage to determine if it may influence microplastic loads.

*Sampling point 1-Vaal Dam*

The Vaal Dam (VD), was the uppermost section of the river tested in this study. Sampling in the dam took place on the UJ Island Reserve, approximately 3.5 km North East of the Vaal Dam wall. Sampling took place over two days with different weather patterns, on day one the weather was windless. The second day was dominated by an easterly wind that blew towards the shoreline and flooded the shore with high amounts of algae. Microplastic levels were expected to be lowest here in the water with increased levels in the sediment due to the still-standing water.

*Sampling point 2- After Dam Wall*

After Dam Wall (ADW) was located approximately 1.5 km downstream of the Vaal Dam wall. The stretch of the river where sampling took place appeared to have some human impact with residential houses on the eastern bank. The water was teeming with life in the forms of aquatic invertebrates that were observed in the water. The section of the river was extremely deep, being >2 m in depth just over a meter from the shore. Microplastic levels were expected to be increased in water and decreased

in sediment here due to the increased flow from the Vaal Dam which constantly releases water into the river.

*Sampling point 2- Loch Vaal Club*

Loch Vaal Club (LVC) was located within the Vaal River Barrage approximately 2 km upstream of the Vaal River Barrage weir. The weather varied over the two days of sampling with a distinct westerly wind blowing towards the shoreline on the first day where sampling took place with no wind on the second day. The section of the loch was characterised by deep water similar to ADW, sampling point 2, but with no flow. Aquatic life activity was abundant in the area, indicating ample resources for feeding. Here, microplastic pollution was expected to increase compared to the Vaal Dam, as more pollution from runoff enters the river before this section. Although the deep still-standing water could affect the microplastic loads in the sediment and water.

*Sampling point 4- Vaal Rus*

Vaal Rus (VR), was located approximately 4.7 km downstream of the Vaal River Barrage. The weather over the two days of sampling remained constant with no extreme weather patterns. The water was incredibly polluted with algae and other indications of possible sewage runoff. The shoreline depth was similar to that of VD. Here microplastic loads were expected to increase as it was presumed the most polluted section of the river, however, sections of the river that consisted of massive stones and boulders may influence sediment microplastic loads.

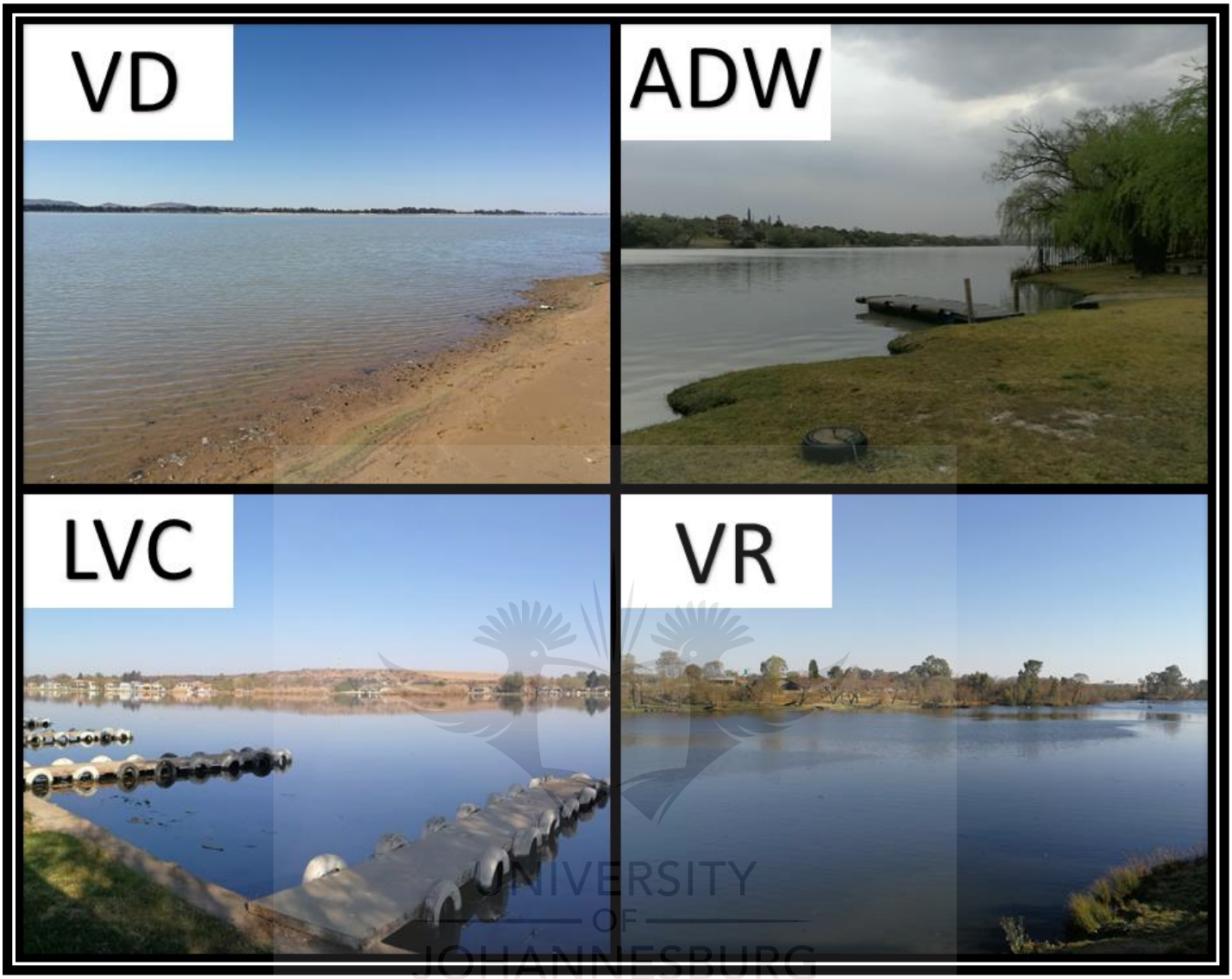




Figure 3: Photos of the upper Vaal River where sampling took place. Sampling points follow downstream from Vaal Dam (VD) to After Dam Wall (ADW), Loch Vaal Club (LVC) and Vaal Rus (VR), the site furthest downstream. VD represents the Vaal Dam and LVC the Vaal River Barrage. ADW is located after the Vaal Dam wall and VR follows after the Vaal River Barrage.

## 2.3 Experimental design

Microplastics are researched within three primary matrices of an aquatic ecosystem. Many studies that have been published will either focus on the water column, sediment or a bioindicator organism with research primarily focusing on one or two of these aspects (Rochman et al. 2015; Ory et al. 2017; Lehtiniemi et al. 2018; Li et al. 2018; Collicutt et al. 2019). Some researchers have started to not only determine microplastic levels or its presence but have investigated its means of transport through abiotic factors such as wind, streamflow, ocean currents and depth (Peng et al., 2018; Rezaei et al., 2019; Kane et al., 2019; 2020; Weideman et al., 2020). This research project aimed to investigate all three of these matrices. Due to the lack of a consistent method applied over all microplastic studies to assess the presence of microplastics, a methodological review was applied in a study on the microplastic profile of the Braamfontein Spruit (Hidalgo-Ruz et al., 2012; Li et al., 2018). The adapted method in this study would then be applied in the Vaal River. Due to certain influences in the Vaal River such as the Vaal Dam wall and Vaal River Barrage weir, similar obstructions and structures were investigated in the Braamfontein Spruit to further understand how certain environmental characteristics could influence microplastic contents in the Vaal River.

Analysing samples for microplastics can vary from a simple and quick process to hours of hard work depending primarily on the sample and how it was prepared for analysis. Water, sediment and biota have different methodologies used in various studies, but the main goal of the experiment is simple, remove as much organic and inorganic matter as possible to ease the visual identification of the plastic (Hidalgo-Ruz et al., 2012; Lusher et al., 2017; Li et al., 2018; Collicutt et al., 2019). Visual identification still plays the key role in microplastic research with or without the use of more sophisticated methods. These would include analysis such as µ-Fourier Transform-Infrared Spectroscopy (µ-FT-IR) or Raman Spectroscopy that analyse the polymer of the plastics collected, which can be used to accurately determine if a material is a plastic and counted correctly (Hidalgo-Ruz et al., 2012; Lusher et al., 2017; Li et al., 2018).

Visual identification using a stereo and light microscope remains problematic. Microplastics can be hard to identify without practice and proper experience with microscopy (Lusher et al., 2017). Guides such as the Marine and Environmental

Research Institute (MERI) “Guide to microplastic identification” can aid in microplastic identification but further knowledge, research and training is required in the art of identifying microplastics (MERI, 2015).

Various studies have found that the accuracy of visual identification can differ due to differences in the reader, methods and most importantly the size, colour and type of microplastics contained in the sample (Hidalgo-Ruz et al., 2012). In this study, to accurately identify microplastics using visual identification, a checklist was followed similar to what were described by Hidalgo-Ruz et al. (2012), who was similarly referred to in the microplastic identification guide of MERI. Particles that were identified had to conform to the following set of criteria to be classified as a microplastic:

1. No cellular structure could be identified (fibres and filaments had to be assessed further by light microscopy).
2. Fibres and filaments had to be evenly thick.
3. Objects that easily broke apart when gently pressed with a dissection needle were not counted
4. Materials that felt like or had a glass-like texture were not counted
5. One in three particles were tested with the hot needle test, where a needle is placed over a flame until red hot and gently moved past the particle, without making contact. If a particle moved or curled to the hot needle it was accepted as a plastic particle.



If the material identified had not met these criteria, or if any uncertainty of whether the material was plastic or not occurred, then the material was not counted, to determine a conservative estimation of microplastics in the environment. Studies have found that visual identification of microplastics in the size range 5 mm-0.5 mm may be highly accurate, however, smaller plastics may present more of a challenge and reduce the accuracy of identifying microplastics (Lusher et al., 2017).

The final challenge remaining in microplastic studies is contamination during laboratory assessment. With microplastics occurring in various forms in the dust, from clothing and in the air, great care must be taken when microplastic analysis is undertaken (Hidalgo-Ruz et al., 2012; Lusher et al., 2017; Rezaei et al., 2019). It is therefore essential to eliminate and identify any possible contamination of samples from airborne microplastics. Glassware used in the study underwent a vigorous soap

and acid bath cleaning process before use (Giesy and Wiener, 1977). All glassware, equipment and work surfaces were washed and rinsed with distilled water several times before use. During identification, Petri dishes remained closed and all samples were covered with aluminium tinfoil. A dark black lab coat was worn through the laboratory analysis and any fibres resembling the lab coat were removed from the sample and not counted. Movement in the laboratory when microplastics were counted was restricted, to reduce any potential transportation of fibres in the workspace. Finally, at the end of counting, blank controls with distilled water were counted and the number of plastics collected were deducted across all the samples.

## 2.4 Conclusion

Microplastics are an emerging contaminant, regarding scientific output, that presents several challenges to researchers from finding and identifying to correctly quantifying plastic pollution within a system. Although great strides have been made in microplastic research, a lack of understanding with regards to not only their effects but how they behave in the ecosystem leaves the need for more intensive research and monitoring in the environment. This study aims to indicate some of these areas that require more research. The Braamfontein Spruit, a smaller stream in a highly-populated area in the biggest city in South Africa and the upper Vaal River, the work-horse of South Africa provide not only the perfect areas to study the role of microplastics in the freshwater ecosystems but to determine the need for better plastic waste management in South Africa.

# Chapter 3

# The microplastic profile of an urban African stream



Reference

Dahms, H.T.J., van Rensburg, G.J., Greenfield, R., 2020. The microplastic profile of an urban African stream. Sci. tot. Env. 731. 138893. https://doi.org/10.1016/j.scitotenv.2020.138893

Note*

The following chapter, “The microplastic profile of an urban African stream”, has been published in the peer reviewed journal “Science of the Total Environment”. The chapter has been modified from the published version. Author permission declaration can be located in Annexure 3.

# Chapter 3

## 3.1 Introduction

Microplastic pollution has been documented globally, on every continent and in every ocean, from the polar regions to the equator and from the ocean surface to the deepest abyss (Rochman et al., 2015; Blettler et al., 2018; Eerkes-Medrano and Thompson, 2018; Li et al., 2018; Peng et al., 2018). Microplastics are defined as plastic particles between 0.05-5 mm in size (Arthur et al., 2009). They enter the aquatic environment either directly through products that contain microplastics or are transported from the terrestrial environment by biotic and abiotic factors (Ory et al., 2017; Li et al., 2018). Microplastics are then further defined as either primary microplastics (plastic beads used in air blasting and cosmetic products) or secondary microplastics (plastic broken down from larger pieces of plastic such as shards and filaments, Li et al., 2018).

Currently, there is an exponential growth of microplastic research around the world since the first detection made around the coast of New Zealand in 1977 by Gregory (1977). However, there is a distinct lack of research papers on the presence of microplastics in Africa (Blettler et al., 2018; Eerkes-Medrano and Thompson, 2018; Li et al., 2018; Peng et al., 2018; Reed et al., 2018). Little is known about the presence of microplastics in the South African freshwater environment and how it can affect the ecosystem (Naidoo et al., 2015; Verster et al., 2017). A recent study on microplastics in the Orange-Vaal River system indicated that large dams may not concentrate microplastics in high quantity in the water column itself, however, sediment and living organism microplastic abundances were not tested (Weideman et al., 2019). South Africa is a developing country with an estimated domestic plastic consumption of approximately 1.8 million tonnes in 2017 alone, of which almost 1.5 million tonnes being virgin materials (Plastics SA, 2017). The South African plastic market was estimated to be worth 67 billion South African Rand (4.8 billion US dollars) in 2017 (Plastics SA, 2017). From 2005 to 2017, South Africa had consumed approximately 20 million tonnes of virgin plastic (Plastics SA, 2017). Only a small fraction of the total plastic used each year is from recycled plastics. In 2017 an estimated total of 300 000 tonnes of recycled plastic was used compared to 1.5 million tonnes of virgin plastic, therefore a substantial amount of plastics could enter the environment in South Africa

(Plastics SA, 2017). These facts coupled with the raw sewage disaster in the Vaal River system as reported by Hosken (2018), indicate that South African rivers may be experiencing a microplastic disaster, as sewage may contain billions of microplastics as highlighted by Li et al. (2018).

Field studies on microplastic pollution have focused mainly on marine environments with only 13% being on freshwater environments and a limited number of these being conducted in urban streams in highly populated cities (Blettler et al., 2018; Eerkes-Medrano et al., 2018; Li et al., 2018). Freshwater microplastic research has primarily focused on one or two of the biotopes of rivers, lakes or coastal areas and may include a few species found there (Rochman et al., 2015; Ory et al., 2017; Lehtiniemi et al., 2018; Li et al., 2018; Collicutt et al., 2019; Syakti et al., 2019). Few studies compare organisms and multiple biotopes to establish any relationships on streamflow, microplastic deposition and the intake of these plastics by an organism (Nel et al., 2018; Collicutt et al., 2019). Some of these studies have collected and analysed microplastics for toxins absorbed by the plastics and have found organic pollutants such as dichlorodiphenyltrichloroethane (DDT) and metals such as mercury, cadmium and uranium in concentrations several orders of magnitude higher than the surrounding environment (Collicutt et al., 2019; Guo and Wang, 2019). Microplastics could be highly concentrated point sources of toxins when ingested by animals, including humans (Peixoto et al., 2019; Rainieri and Barranco, 2019). One such family of organisms is the Chironomidae (common midges), which have a global distribution. The larval stage of the Chironomidae are deposit feeders, found in many types of aquatic habitats, thrive in hypoxic environments and are relatively tolerant to anthropogenic stressors, making them suitable indicator organisms (Nel et al., 2018).

This chapter aims to provide a single snapshot of the microplastic profile of an urban stream that passes through various urban green zones in Johannesburg, South Africa. The profile will be assessed by defining the number of microplastics in surface water, benthic macroinvertebrates (*Chironomus* spp.) and stream sediment. *In situ* stream characteristics will then be analysed to establish if they influence the microplastics profile. The study could provide the first indication of microplastics in the streams around Johannesburg. It is hypothesised that (i) microplastics will be detected in water, sediment and macroinvertebrates in the Braamfontein Spruit; (ii) microplastic occurrence is influenced by stream characteristics like stream velocity, depth and

man-made structures and (iii) benthic aquatic macroinvertebrates will show a similar microplastic accumulation trend as the sediment that they inhabit.

## 3.2 Method and materials

### 3.2.1 Study sites

Nine sites along the Braamfontein Spruit (Figure 4) were selected for this study. Sampling of all the sites was completed within one day (25 June 2019) to provide a single snapshot of the microplastics profile of the stream while avoiding the influence that weather patterns may have. The Braamfontein Spruit flows through several suburbs and connects various green zones around Johannesburg the largest city in South Africa. The Braamfontein Spruit is joined by two large tributaries the Westdene and Montgomery Spruits before it finally joins the Jukskei River, a tributary of the Limpopo River (City parks, 2014). The Limpopo River is a transboundary river to multiple African countries and is an important water source for agricultural land uses and subsistence fishermen in Southern Africa (City parks, 2014). The nine sites were selected based on various stream characteristics and surrounding activities that cover the length of the stream. This was to understand how blockades such as a large weir and shallow rapids could have on the microplastic dispersion.

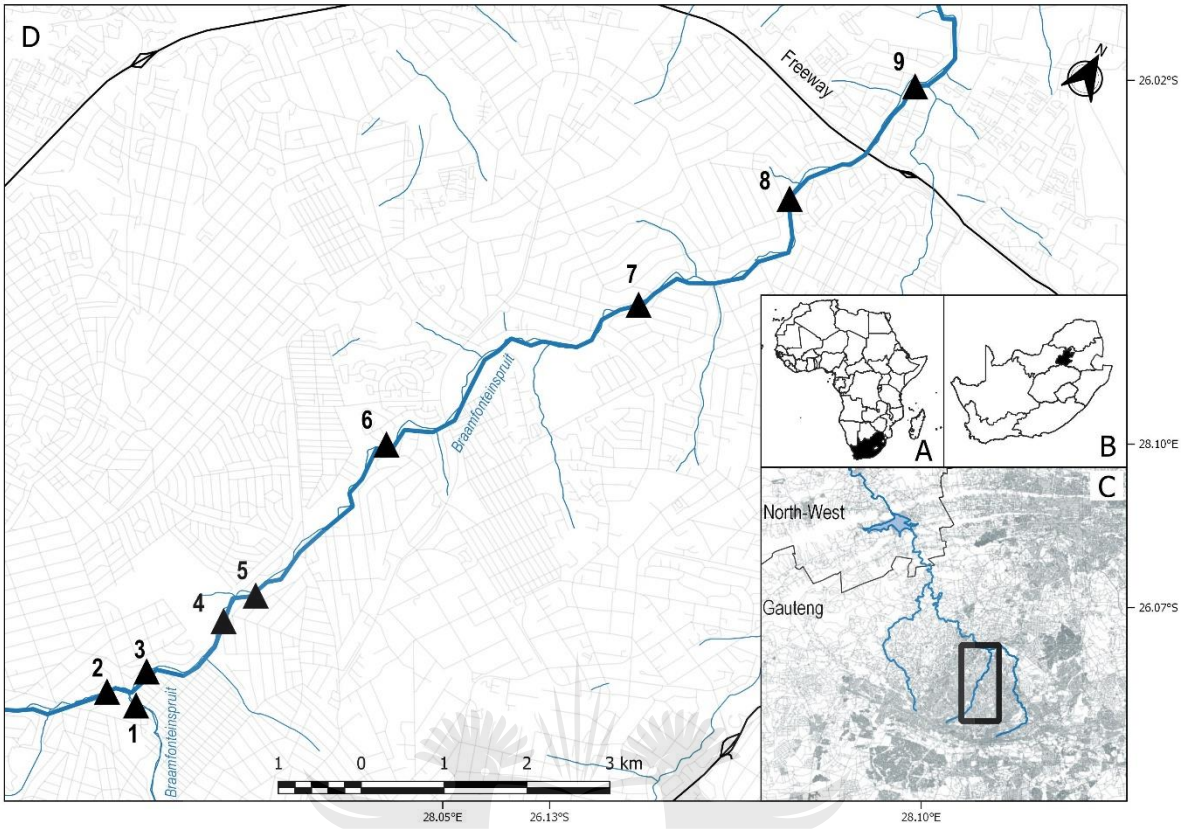


Figure 4: Map of selected sites along the Braamfontein Spruit (D). Map inserts indicating the location of South Africa (A), Gauteng province (B) and the urbanisation surrounding the study area (C).



### 3.2.2 Sample collection

#### 3.2.2.1 *In situ* parameters

To establish any possible relationships of microplastic abundances and stream characteristics a large assortment of *in situ* parameters were taken. Water clarity was recorded where water samples were collected, depth of water was recorded where sediment was collected (sed depth) and water velocity recorded where both sediment (sed velocity) and water (water velocity) samples were collected. Clarity was measured using a clarity tube and velocity and depth measured using a transparent velocity head rod, according to the Rapid Habitat Assessment Methodology (RHAM) prescribed by the Department of Water and Sanitation (DWAF) (DWAF, 2009).

### 3.2.2.2 Water collection

Water was sampled following the method of Collicutt et al. (2019) with modifications. Water was collected four times in a 25 L container at different longitudinal points of the site (100 L in total per site) for a better representation of microplastics found in the stream at the particular sites. The container was placed in the stream close to the surface with the opening faced towards the oncoming flow of the stream with the researcher downstream of the container to prevent contamination from the researcher's clothing. The water was then filtered through a series of stainless-steel sieves (4000, 212 and 53 µm) to capture microplastics and eliminate larger debris such as plant material that may have been collected. Larger biological material was rinsed, removed, and the remaining material collected was rinsed into a 50 mL container for laboratory analysis. The sieves and containers were rinsed multiple times at each site to prevent any cross-contamination from various sites. To allow for standardisation across all sites, the contents of the sieves were rinsed into the plastic container three times.

### 3.2.2.3 Invertebrate collection

*Chironomus* spp. larvae were caught using a 1 mm mesh size net and a kick-stir-sweep method (Dickens and Graham, 2002). *Chironomus* spp. larvae were identified in an identification tray, placed in 50 mL plastic containers and immediately killed by adding 10% neutrally buffered formalin. Time of sorting and rapid euthanasia was considered important to prevent the ejection of gut contents which could skew results as detected by Nel et al. (2018). *Chironomus* spp. larvae were sampled last to prevent any microplastics in the sediment to become suspended and contaminate water microplastic samples. A minimum of 75 individual larvae were collected at each sampling site.

### 3.2.2.4 Sediment collection

Sediment was collected after the collection of water microplastic samples to prevent contaminating water microplastic samples by removing approximately 2 kg of top sediment in the river (≈ 10 cm depth). The sediment was placed in a zip lock bag and returned to the laboratory (Nel and Froneman, 2015; Collicutt et al., 2019).

### 3.2.3 Laboratory analysis

#### 3.2.3.1 Sediment characteristics

Sediment samples were weighed and dried in an oven at 50 °C for three days to determine the dry weight. A subsample of dried sediment was then used to determine the particle size and organic content of the different sites. Particle size was determined using a mechanical sieve system with sieve sizes of 4000 µm, 2000 µm, 500 µm, 212 µm and 53 µm. Approximately 100 g of dried sediment was sieved and after shaking for 10 mins each constituent was weighed to get a percentage of the sediment profile of each study site which was classified as described by Cyrus et al. (2000), (Table 2). Organic content was determined according to the United States Environmental Protection Agency (USEPA), (2001) and American Society for Testing and Material Standards (ASTM), (2000) technique. Approximately 2 g of dried sediment was accurately weighed for each site and placed in porcelain crucibles. The samples were then incinerated at 600 °C for 6 hours to remove any organic matter. The samples were weighed after incineration to determine the organic content percentage for each sample.

#### 3.2.3.2 Microplastic extraction from water

Collected water samples were placed in glass beakers that were covered by a piece of aluminium foil. The volume was measured, and the correct amount of potassium hydroxide (KOH) was added to create a 10% KOH solution for the digestion of most organic matter but not any plastic material (Gómez-Hernández, 2012). The water samples were then digested at room temperature for approximately 18 hours (Gómez-Hernández, 2012). The contents were then placed covered in a clean glass petri dish for microscope analysis. Samples were immediately analysed, covered with the glass petri dish top, to prevent any airborne contamination.

#### 3.2.3.3 Microplastic extraction from *Chironomus* spp. larvae

*Chironomus* spp. larvae were washed with distilled water to remove any external microplastics (Nel et al., 2018). The larvae were then divided into four groups of 15 individuals and the mass of each replicate was determined and later used for

standardization, number of plastics per gram wet weight (ww). The organisms were then placed into small microcentrifuge tubes with 1.9 mL of 10% KOH solution for three days to digest the organic matter (Gómez-Hernández, 2012). The organisms were then gently crushed and vortexed to break the remaining exoskeleton and release any remaining stomach contents, similar to the method followed by Windsor et al. (2019). The solution was then placed in a covered glass petri dish for microscopic analyses.

### 3.2.3.4 Microplastic extraction from sediment

Sediment was dried at 50 °C for three days until dry. A 500 g dry weight (dw) subsample was then taken from the dried sediment for microplastic enumeration and identification through density separation (Nel and Froneman, 2015; Coppock et al., 2017; GESAMP, 2019). A review study by Coppock et al. (2017), found recovery rates of 99% for large microplastics (1-5 mm) and 40-72% for smaller microplastics (<1mm) in similar methods tested by Imhoff et al. (2012). The sediment was then placed in large one-litre glass beakers that were filled with a hypersaline NaCl solution (339 g $L^{-1}$) until the sediment and salt solution reached approximately 500 mL (Coppock et al., 2017). The sediment-salt solution was then stirred vigorously for 2 min with a cleaned metal rod, the top covered with aluminium foil and placed on an orbital shaker for 18 hours to dislodge any microplastic particles (GESAMP, 2019). The hypersaline solution allows for the less dense microplastics to float to the top of the solution while the heavier sediment remains at the bottom of the beaker resulting in a high recovery rate of microplastic material (Hidalgo-Ruz et al., 2012; Coppock et al., 2017; GESAMP, 2019). The sediment-salt solutions were then left for 6 hours to allow any sediment to settle and microplastics to rise (Coppock et al., 2017; GESAMP, 2019). The liquid was then washed through a series of stainless-steel sieves (4000 and 53 µm) to remove large and smaller particles. The hypersaline solution was added three times to the samples and washed into the sieves to remove any possible microplastics in the sediment samples (Hidalgo-Ruz et al., 2012; Coppock et al., 2017; GESAMP, 2019). Similarly, any material that was caught on the sides of the beaker was rinsed back into the beaker with the hypersaline solution and washed into the sieves. The contents on the sieves were then washed three times into a glass petri dish with distilled water for microplastic identification and enumeration.

### 3.2.3.5 Microplastic identification

All samples were placed in clean, rinsed glass Petri dishes and identified and enumerated using a Carl Zeiss Stemi DV4 dissection microscope. The microplastics were visually identified based on their shape and colour (Rochman et al., 2015; Windsor et al., 2019). Identification was based on certain characteristics as stated in the MERI's guide to microplastic identification (2015), examples in Figure 8, that closely followed the step by step guide established by Hidalgo-Ruz et al. (2012). Objects that contain any cell structure were not counted, filaments had to be evenly thick and single colour. Clear or transparent filaments had to be assessed on a light microscope and if an object broke apart or had a glass-like texture when pressed with a needle it was also excluded (Hidalgo-Ruz et al., 2012). A hot needle test was then performed to establish if the object was a possible plastic polymer if the objects curled or compressed due to the hot needle being close to the object but not coming into contact with it, it was counted as a microplastic particle (Gómez-Hernández, 2012; Hidalgo-Ruz et al., 2012; Lusher et al., 2017). A minimum of one in every three identified particles or any material that could not be identified as a plastic polymer was tested with the hot needle test, which adhered to the requirements of Lusher et al. (2017). If an object failed any of these requirements, or if there were any doubt during its identification due to the size being <0.5mm, it was not counted to establish a conservative number of the microplastic found in the stream.



### 3.2.4 Contamination control

Contamination prevention procedures were adapted from MERI (2015), as well as the procedures stated by Coppock et al. (2017) and Lusher et al. (2017) as contamination control remains a key part of any microplastics study. Glassware was used as containers as much as possible during the study. All tools and storage containers were washed through a soap and acid bath before the study and rinsed several times with distilled water to prevent contamination (Giesy and Wiener, 1977). Workbenches were cleaned and movement around the area where microplastics were read was minimised. All containers were covered with aluminium foil to prevent any airborne contamination. During the microscopic analyses, glass petri dishes were used and rinsed several times before any solution was added for counting. The Petri dish was

kept closed as much as possible and was only opened to remove microplastic particles or to test them to prevent contamination from clothing worn by the researcher. A dark black polycotton lab coat was worn and purple nitrile gloves were worn during the analyses for ease of contamination identification. Any material that resembled the lab coat, clothing or the containers used, was not included in the results section to provide a conservative estimation on the number of microplastics found (Baalkhuyur et al., 2018). To establish the presence of any contamination, all samples had one blank control of distilled water that closely followed the steps for processing each matrix. The distilled water control for the water analysis was placed in the same type of containers and was similarly transferred as the sampled water to assess any possible contamination that might have occurred during the digestion and reading processes. Invertebrates were rinsed before digestion and four blank microcentrifuge tubes with distilled water were also analysed for any contamination. A blank dish was added in the oven when sediment was dried. Due to the sediment having to be uncovered in the drying proses, they were the samples most likely to be contaminated. The oven was therefore closed and only opened once to prevent airborne microplastics from contaminating the samples. The surface was rinsed into a 1 L beaker to establish any contamination during the drying, shaking, filtering and microscopic analyses. Any contamination that was found in the blank samples was subtracted from all relevant samples that could similarly have been contaminated for all matrices tested.



### 3.2.5 Statistical analysis

GraphPad Prism (Prism 5 v.5.03) was used for the construction of bar graphs. Multivariate analysis was performed to get a more holistic view of the data spread. A constrained ordination of sediment depth, sediment velocity and water velocity on the types of microplastics across all the sites was investigated through a redundancy analysis (RDA, Canoco v.5). A square root transformation was applied to the types of microplastic counts to mitigate for extremely high counts while accounting for the zero counts of some types. Site and microplastic distribution maps were created with QGIS v 3.10.8.

## 3.3 Results

### 3.3.1 *In situ* parameters and sediment characteristics

*In-situ* parameters are presented in table 1. Stream depth varied among the sampling sites. Site 2 had the deepest overall water depth (0.33 m) and site 8 the shallowest (0.07 m). Stream velocity where sediment was sampled remained below 0.12 m $s^{-1}$ except at site 5 where it increased to 0.23 m $s^{-1}$. The velocity of the stream where water samples were collected was highest at site 8, 1.03 m $s^{-1}$. Sites 2, 3, 4, 7 and 10 had a velocity of below 0.12 m $s^{-1}$. Clarity remained similar (0.9-0.8 m) throughout all sites apart from site 8 which had a large decrease (0.58 m). Sediment particles and organic content are shown in Figure 5. Sediments tended to be coarse sand with at least 53.8% of the particles between 500-2000 µm in all sites. Site 2 was dominated by medium grains of sand to mud (74% of the particles being <500 µm) and site 4 consisting of coarse sand and gravel (52% of the particles being >2000 µm). Site 2 had high organic content (>7%), while site 6 had the lowest organic content (<1%). The average organic content of the stream can be classified as moderately low although sites 2 and 3 were classified to have a medium to high organic content based on the guidelines set out by the USEPA (1991).

**Table 1: In situ parameters measured at each site in the Braamfontein Spruit**

| Site | Clarity (m) | Water Velocity (m $s^{-1}$) | Sed velocity (m $s^{-1}$) | Sed Depth (cm) |
|---|---|---|---|---|
| 1 | 0.91 | 0.92 | 0.06 | 28.0 |
| 2 | 0.90 | 0.06 | 0.06 | 33.0 |
| 3 | 0.93 | 0.06 | 0.06 | 15.3 |
| 4 | 0.86 | 0.06 | 0.06 | 16.0 |
| 5 | 0.89 | 0.84 | 0.23 | 15.6 |
| 6 | 0.89 | 0.33 | 0.12 | 11.0 |
| 7 | 0.84 | 0.06 | 0.06 | 7.0 |
| 8 | 0.58 | 1.03 | 0.06 | 13.0 |
| 9 | 0.80 | 0.06 | 0.06 | 20.0 |

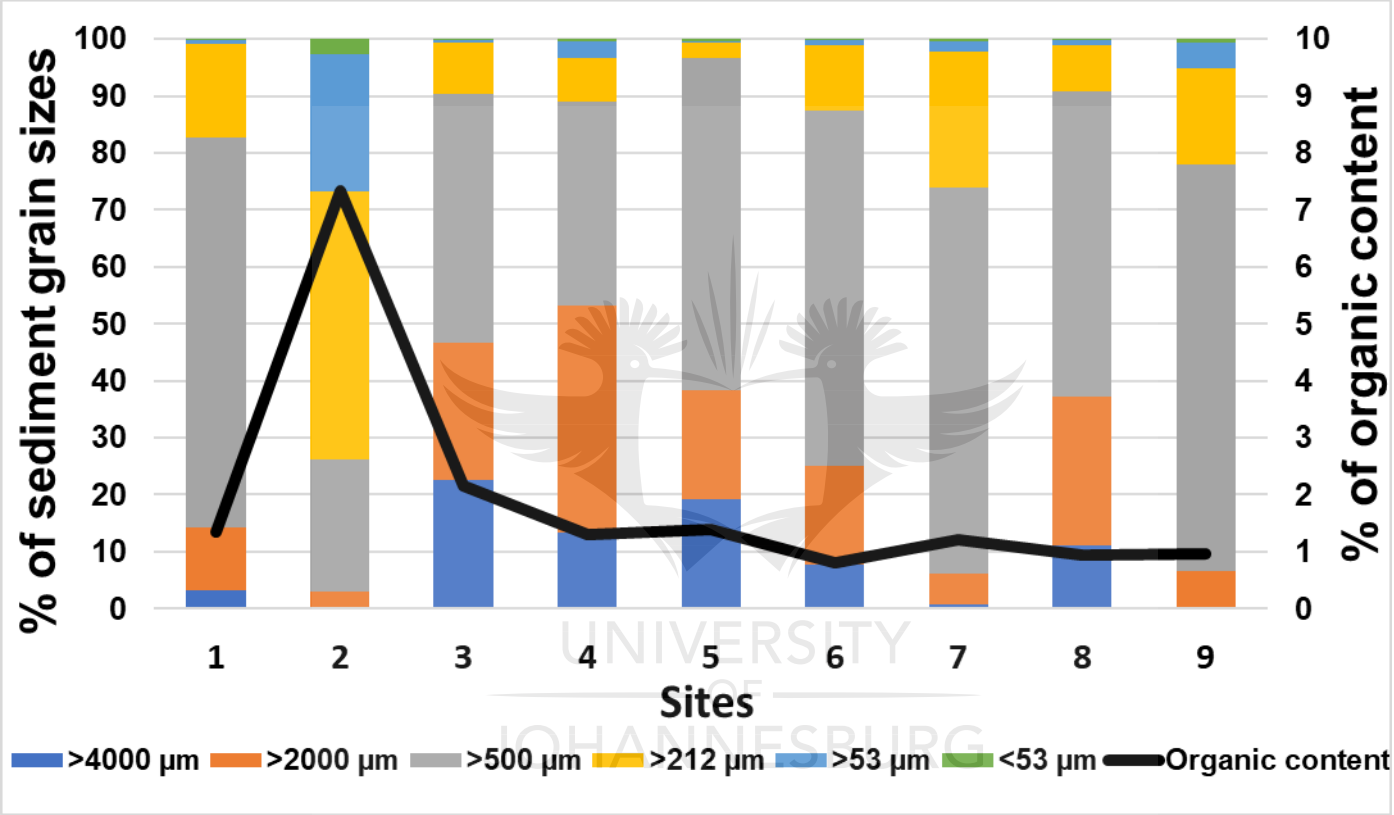


Figure 5: Stacked bar graph indicating percentage sediment grain size composition and organic content of sites selected for the study

**Table 2: Sediment classification as described by Cyrus et al. (2000)**

| Grain size (µm) | Classification |
|---|---|
| >4000 | Gravel |
| 2000-4000 | Very coarse sand |
| 500-2000 | Coarse sand |
| 212-500 | Medium sand |
| 53-212 | Very fine sand |
| <53 | Mud |

### 3.3.2 Microplastics in water

Water samples had a 100% microplastic prevalence along the Braamfontein Spruit (Figure 6A). Site 8 had the highest abundance of microplastics at 2080 particles $m^{-3}$. Site 1 had the lowest abundance with a total of 160 particles $m^{-3}$. The mean abundance of microplastics observed over all water samples was 705 particles $m^{-3}$. Although a high number of microplastics were recorded at site 8 with a steep decrease to site 9, the results indicate that a steady increase of microplastic abundance occurred downstream of the first sampling site. Curiously a decrease in the number of microplastics was found between sites 3 and 4, sites 4 and 5 and sites 8 and 9 (difference of 390, 30 and 1210 particles $m^{-3}$ respectively). Site 3 was located at the confluence of a tributary (site 2) joining the Braamfontein Spruit (site 1), where a steep increase in microplastic abundance was found. Although a large weir was located between sites 4 and 5, it had little effect on the microplastic abundance, only a slight decrease from 450-420 particles $m^{-3}$. Filaments (Figure 7A) dominated in terms of the most prominent shape found in water samples (76.3%), followed by other shaped objects (14.3%), round (6.3%) and angular (2.9%). Transparent/white (30%), blue (29%) and black (21%) dominated the colour schemes, Figure 7B of the microplastics that were found.

### 3.3.3 Microplastics ingestion by invertebrates

The prevalence of microplastics in *Chironomus* spp. larvae groups was 100% throughout all sampled sites (Figure 6B) with at least 1 microplastic being found in each subsample. Site 4 had the highest microplastic abundance (96.7 particles $g^{-1}$

ww) closely followed by site 3 (90.3 particles $g^{-1}$ ww). The lowest abundance of microplastics was found at site 10 (19.8 particles $g^{-1}$ ww). The mean abundance of microplastics found in invertebrates throughout all sites was determined to be 56.2 particles $g^{-1}$ ww. The abundance of microplastics seemed to increase from sites 1 to 4 and sites 6 to 7 before rapidly decreasing. Between sites 4 and 5 a large weir was noted, similarly between sites 7 and 8, a partial gabion was located. Filaments were the predominant shape, Figure 7C, found throughout all invertebrate samples (95.0%), followed by angular (3.6%), other shapes (1.0%) and only one round microplastic (0.4%) was found. The colour scheme, Figure 7D, that dominated most microplastic types was blue (37.4%), followed by black (23.5%), other colours (19.0%), red (13.2%), transparent/white (3.9%) and green (2.9%) being the least prevalent colour scheme.

### 3.3.4 Microplastics in sediment

Microplastics were prevalent in all sediment samples (Figure 7C). Site 2 had the highest abundance of microplastics (1347.5 particles $kg^{-1}$ dw) with site 6 having the lowest abundance (4 particles $kg^{-1}$ dw). The mean number of microplastics throughout the system was 166.8 particles $kg^{-1}$ dw however if the significant influence of site 2 is removed the mean equates to 19.3 particles $kg^{-1}$ dw. The abundance of microplastics in sediment showed an overall decline from site 2 to site 6 (4 particles $kg^{-1}$) after which there was a small increase at site 7 (15 particles $kg^{-1}$). Other shaped objects, Figure 7E, dominated as the most frequent shape found (68%) followed by filaments (19%), with round (11%) and angular (0.9%) the lowest overall. Transparent/white microplastics, Figure 7F, dominated the colour scheme of all particles collected (80%). This was followed by black (7.9%), blue (5.7%), other colours (4.9%) and green (0.3%).

### 3.3.5 Contamination control

Control samples ran concurrently with all samples tested in this study and followed the exact procedure from sampling, laboratory analysis and counting. Water samples showed to have no contamination as it had the least amount of preparation compared

to sediment and invertebrates. The invertebrates had a total of 1 transparent/white microplastic filament and the sediment controls a total of 5 filaments

### 3.3.6 Redundancy analysis

An RDA (Figure 9) was used to investigate the relationships between certain environmental variables and the abundance of the types of microplastics. Axis 1 explains most of the variation in the data (97.21%). An increase in water velocity was most strongly associated with the microplastic types identified in the water column, particularly filaments. An increase in the water depth at which sediment was sampled (sed depth) was strongly associated with microplastics in both invertebrates and sediment, particularly the angular type. Water velocity over the sediment (sed velocity) showed a strong inverse relationship with a few of the samples, mainly filaments in invertebrates, indicating that where the water had higher flow there was a reduction in the amount of filament type particles.

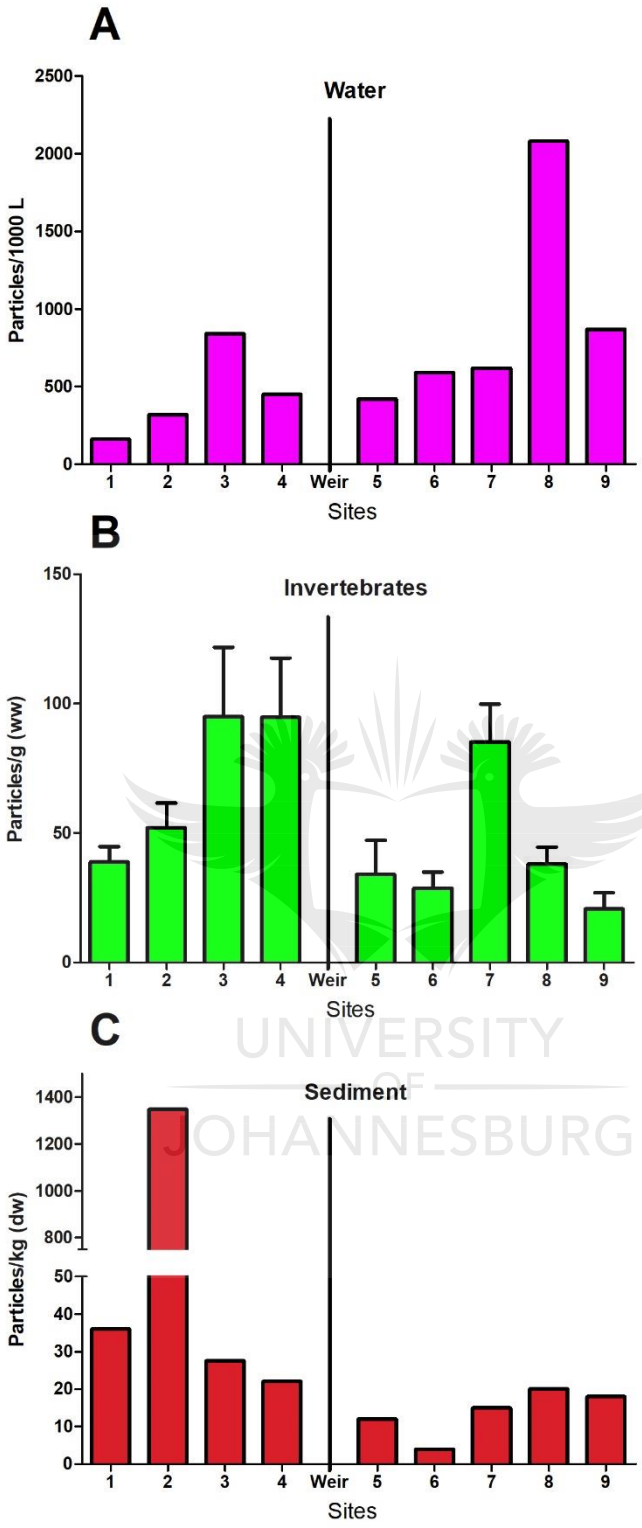


Figure 6: Bar graphs of the total number of microplastics found in water (A), mean number in invertebrates (B) and sediment (C). ww – wet weight, dw – dry weight.

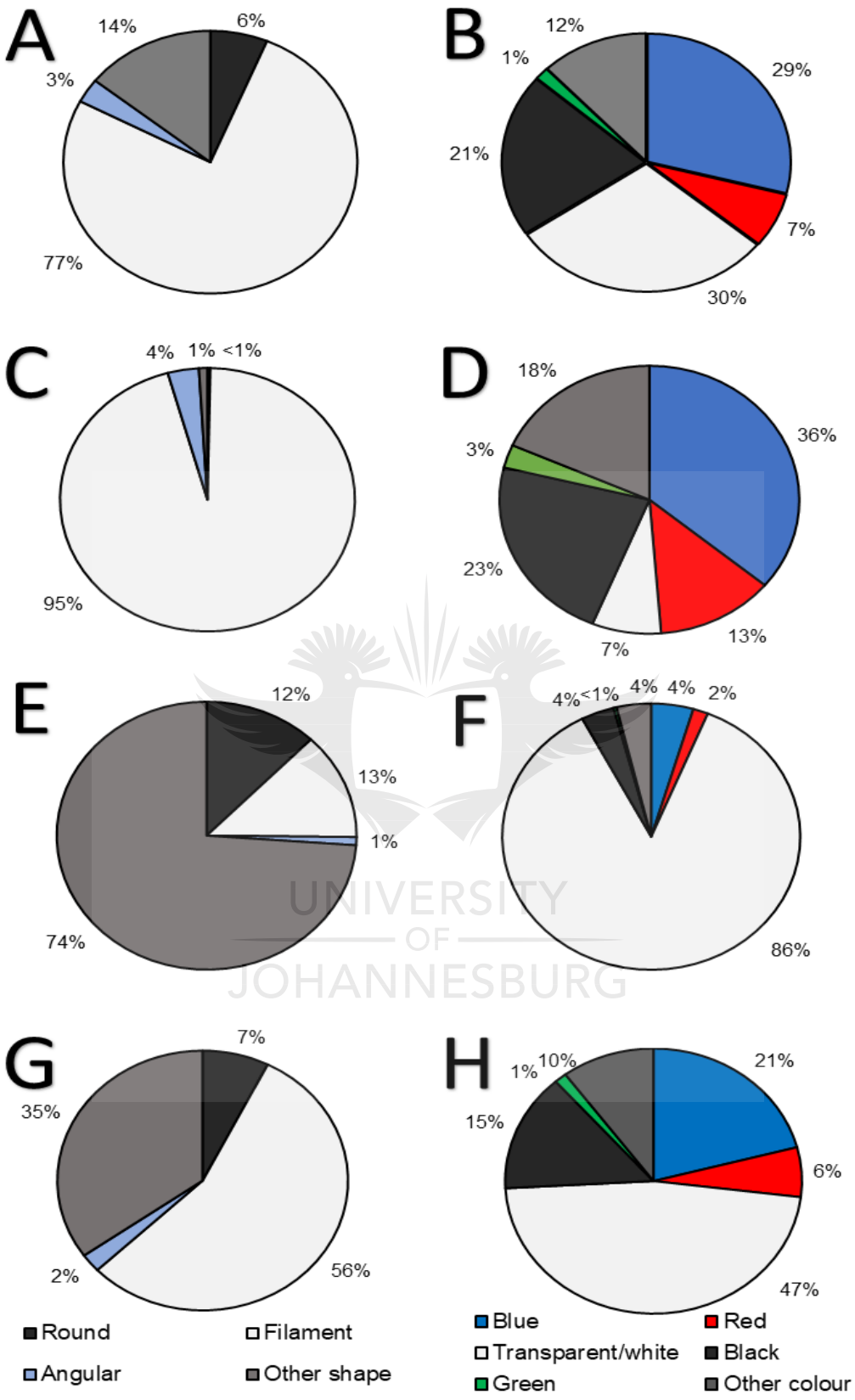


Figure 7: Pie charts illustrating the different forms and colours of microplastics collected in A (forms in water), B (colours in water), C (forms in *Chironomus* spp.), D (colours in *Chironomus* spp.), E (forms in sediment), F (colour in sediment), G (all forms in total) and H (all colours in total) in the Braamfontein Spruit

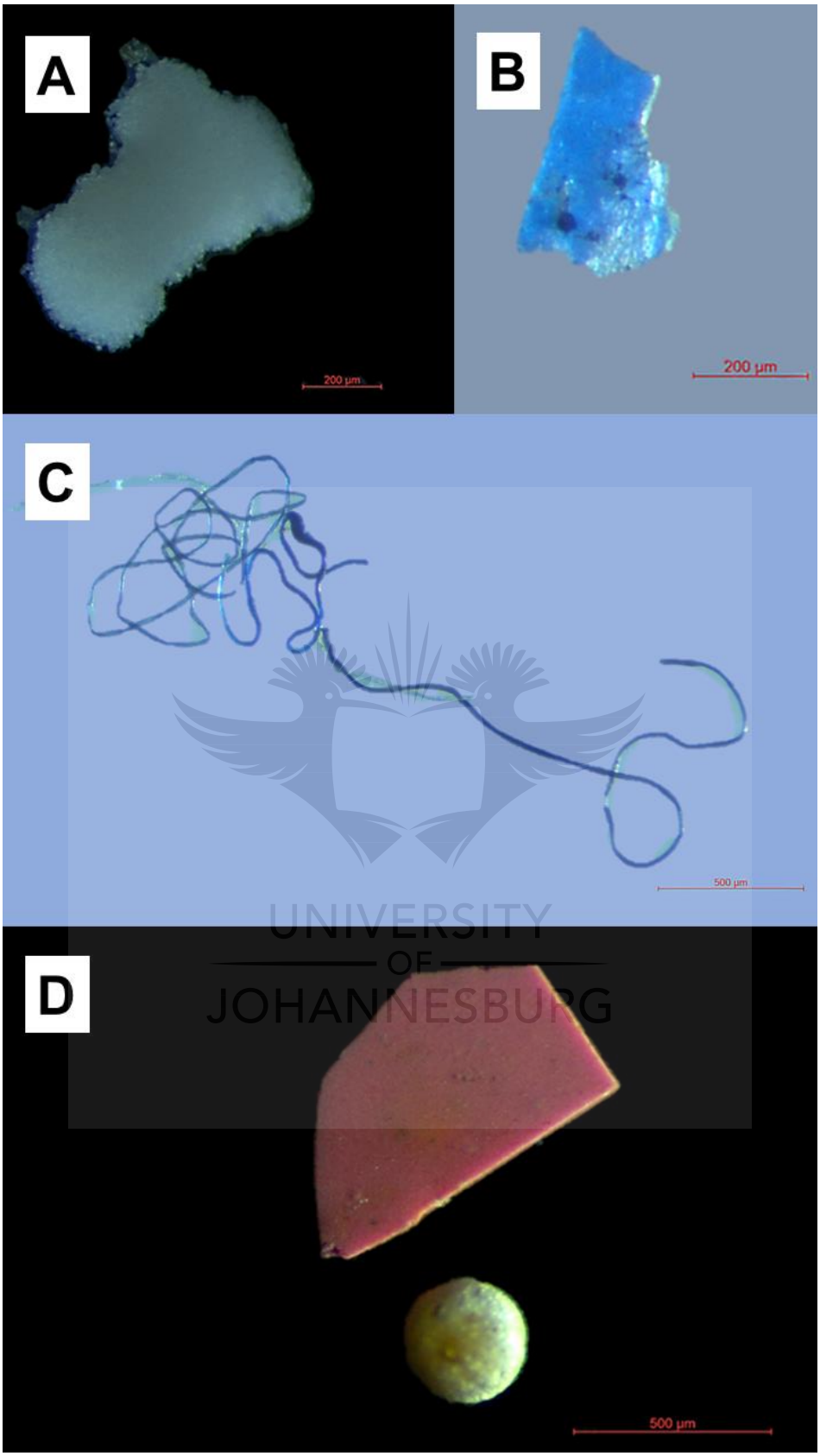




Figure 8: Examples of microplastics collected in the Braamfontein Spruit. A- foam, B- other shaped, C- filaments and D- angular top and round bottom.

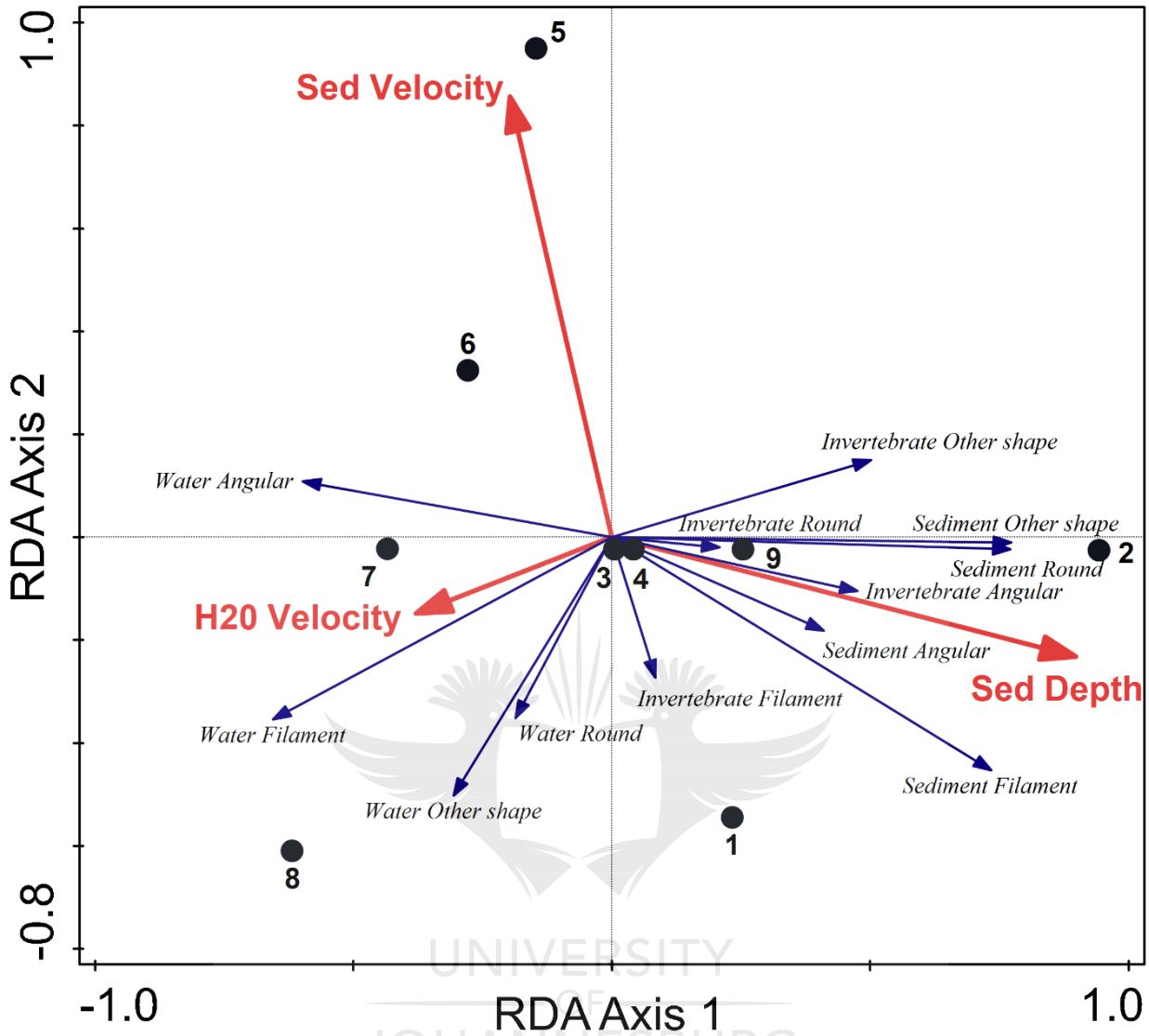


Figure 9: A RDA constrained ordination summarizing the effect of stream characteristics, sed depth (water depth where sediment was collected), sed velocity (water velocity where sediment was collected) and water velocity (velocity of stream where water was collected) (red arrows) on the spread of the types of microplastics in each matrix (blue arrows). The explained cumulative variation over the two axes is 99.24% with 97.21% on the first axis and 2.03% on the second axis.

## 3.4 Discussion

The study has established the presence of microplastics in the water, biota and sediment of the Braamfontein Spruit, an urban stream surrounded by suburbs within a major city in South Africa. This study, as with other microplastic research, can still only establish conservative estimations due to the variety of methods used in establishing microplastic abundances throughout the world. Throughout the study, with the exception of the microplastics in the sediment sample of site 2, the forms of microplastics found were dominated by filaments of various colours. Filaments are secondary microplastics that originate from fishing line and clothing (Li et al., 2018; McIlwraith et al., 2019). Due to the high amount of the South African population living in poverty (over 55% of the population, adding up to over 30 million people), many people make use of rivers and streams for domestic use (StatsSA, 2017). In a study by McIlwraith et al. (2019), it was found that washing one piece of clothing could release thousands of microplastics into wastewater, possibly being the cause for the high filament abundance in the samples.

After the confluence with the Montgomery Spruit, there was a dramatic increase of microplastics in water and *Chironomus* spp. larvae, which indicate that tributaries may play a role in the spread of microplastics to larger rivers and dams, supporting the findings of Nel et al. (2018) and Dikareva and Simon (2019) in the Bloukrans River and urban streams surrounding Auckland, New Zealand, respectively. This, however, was not reflected in the sediment counts with a much larger abundance found at site 2. The finer sediment profile that was found there indicates that finer sediment profiles with higher organic content might allow microplastics to become trapped and more energy would then be required to dislodge them, a similar finding made by Dikareva and Simon (2019). Other evidence for the higher plastics counts at site 2 is provided by the stream characteristics itself as declared in the RDA. The RDA found that water velocity and depth may influence the microplastic distribution, indicating that areas with reduced flow and increased depth may allow microplastics to settle on the bed of the stream which may then be ingested by benthic macroinvertebrates. Similarly, a study conducted by Peng et al. (2018) in the Marianas Trench, a much larger example, found that sediment that was collected in deeper regions of the trench had higher microplastic abundances than shallower areas.

The weir had almost no clear effect in microplastic counts in water as the plastics could simply flow over the obstruction. However, there were differences in sediment, and most notably, *Chironomus* spp. larva with higher abundances upstream of the obstruction and a decreased abundance below it. The microplastic abundances at these two sites coupled with the stream characteristics i.e. an increase in general water velocity after the weir indicate that stream characteristics can have a large impact on the microplastic abundance in different matrices.

From site 7 to site 8, the large increase in water microplastics was associated with an increase in streamflow and a substantial reduction in clarity. An inverse trend was seen for the abundance in invertebrates with a reduction between these sites. Stream characteristics possibly assisted in dislodging and transporting the microplastics in the water from the coarser sediment. However, this could not be a clear contributing factor to such a rapid increase of microplastics in water alone. An anthropogenic factor in the area possibly played a contributory role as signs of wastewater entering the system between sites 7 and 8 was found. A previous study in the United States of America found that wastewater treatment plants could release billions of microplastics into rivers every day, which might indicate why such high levels of microplastics were found at site 8 (Gatidou et al., 2018; Li et al., 2018; Sun et al., 2019; Wang et al., 2019).

A reduction in microplastics from site 8 to 9 was seen in all three matrices. Lower water concentrations may be from the reduction in flow allowing the particles to disperse and settle out well before the site. This would then explain the reduction in invertebrates and sediment where reduced flow over the sediment decreased the abundance in both matrices (similarly between site 5 and 6).

The overall increase of microplastics in water downstream of the origin towards the Jukskei River indicates that streams such as the Braamfontein Spruit can transport microplastics from suburban areas to larger rivers and finally the oceans as declared in similar studies (Nel et al., 2018). Sediment and benthic invertebrate microplastic counts showed a different pattern to water due to changes of environmental factors. This is indicated where a decrease of microplastics downstream were detected in both invertebrates and sediment. What these results possibly indicate is that microplastics enter the stream continuously from external factors, which are transported downstream, as seen in the water microplastic concentrations (Wagner et al., 2014; Mani et al., 2015; Anderson et al., 2016; Nel et al., 2018). Some of these microplastics

are then allowed to settle to the stream bed in areas of reduced flow and increased depth caused by both natural and manmade features. During high rainfall seasons when there is increased flow and greater turbulence in the stream, microplastics trapped in the sediment will be released into the water column which may then be transported downstream in larger concentrations (Nel et al., 2018).

Microplastic research is biased towards different taxa of organisms which are investigated for microplastic ingestion, with studies focusing primarily on fish with little research being conducted on small invertebrates (Blettler et al., 2018; de Sá et al., 2018 Wang et al., 2019; Windsor et al., 2019). The average abundance of microplastics in *Chironomus* spp. larvae was calculated to be 56.2 particles $g^{-1}$ ww with 100% prevalence of the subsampled groups in the Braamfontein Spruit. Few studies have been conducted on any freshwater invertebrates to establish the abundance of microplastics that might be ingested by them. The average of ingested microplastics by *Chironomus* spp. larvae in the Braamfontein Spruit far exceed the abundance that was found in those analysed in the Bloukrans River system by Nel et al. (2018) which was almost five orders of magnitude higher than those found in mussels (0.3 particles $g^{-1}$) by Vandermeersch et al. (2015) and in the lugworm, *Arenicola marina* (1.2-2.8 particles $g^{-1}$ ww) by Van Cauwenberghe et al. (2015). Similarly, the number of plastics was higher than those found in other freshwater African invertebrate species such as *L. varicus* (1.71±0.46 particles $g^{-1}$ ww) in the Osun River in Nigeria (Akindele et al., 2019). The results in this study are however similar to the levels found in *Tubifex tubifex* worms (129-65.4 particles $g^{-1}$ ww) in the River Irwell in England (Wang et al., 2019).

The transfer of microplastics up the food chain in freshwater ecosystems has not yet been confirmed at the point of this study, but it has been well documented in the marine environment and in laboratory studies (Lehtiniemi et al., 2018; Li et al., 2018). The *Chironomus* spp. larvae are low on the trophic system and a food source to possible benthic species, which may be an indication that organisms such as *Chironomus* spp. larvae could ingest sediment microplastics and could lead to more complex organisms ingesting these plastics (Nel et al., 2018). Once ingested, microplastics may have a variety of effects on living organisms. It can cause blockages of the gastrointestinal tract in smaller organisms, lead to reduced growth, movement and reproduction in fish

and may also cause inflammation, cancer, and ultimately the death of the organism (Gatidou et al., 2018; Xiong et al., 2018; Herrera et al., 2019).

The effects of microplastics on organisms could be much more severe than expected, as high concentrations of toxins are absorbed and may release from the surface of the plastics which could then be accumulated by the organism (Collicutt et al., 2019; Guo and Wang, 2019). Unfortunately, the inadequate relationship between laboratory and field studies have caused researchers to expose animals to virgin microplastic concentrations that far exceed environmental levels and are rarely conducted with similar types of plastics such as fibres which were primarily found in this and similar studies (Eerkes-Medrano and Thompson, 2018; Li et al., 2018; Collicutt et al., 2019; McIlwraith et al., 2019)

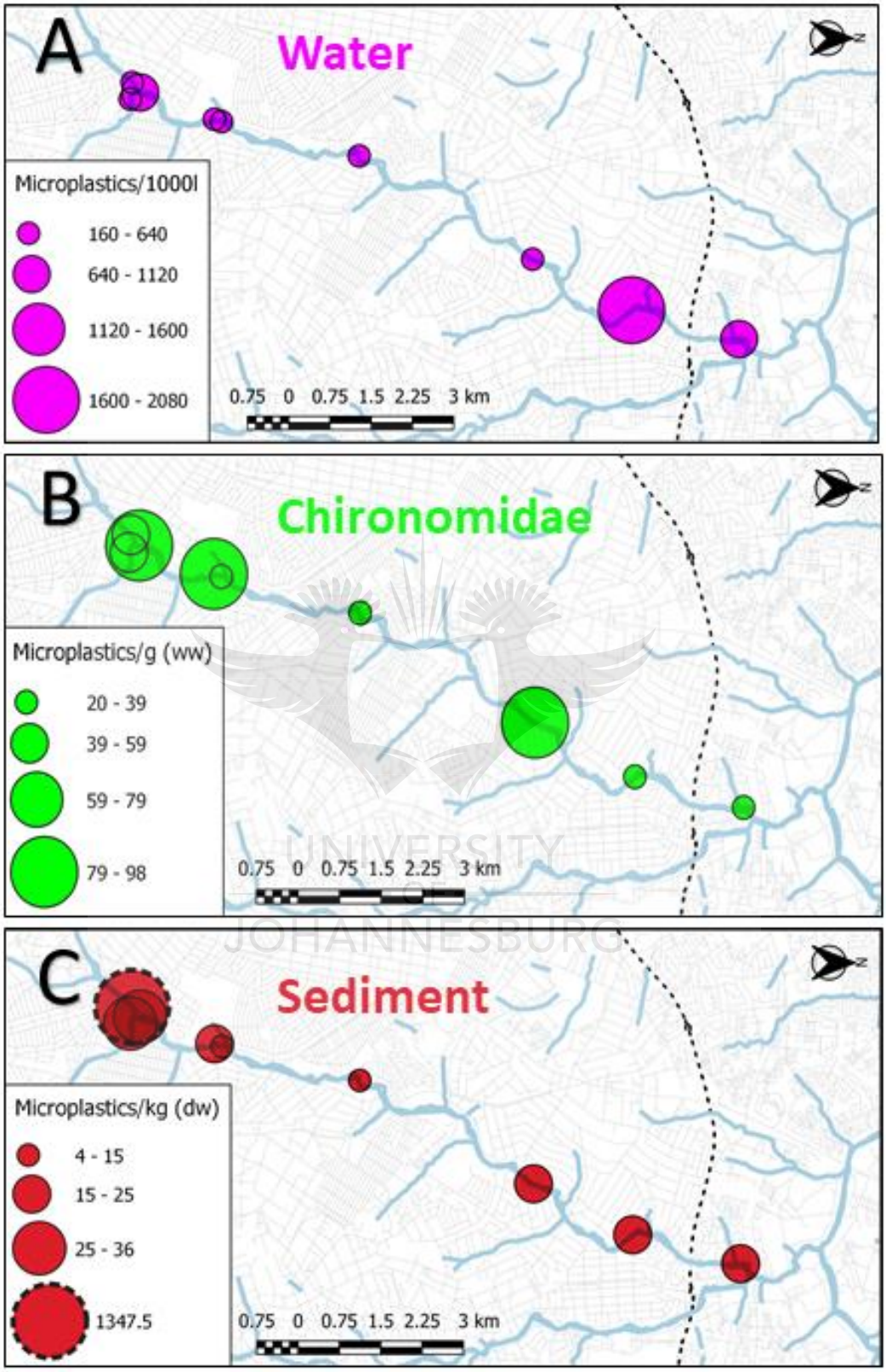


Figure 10: . Microplastic distribution maps of the Braamfontein Spruit in water (A), *Chironomus* spp. larvae (B) and Sediment (C).

## 3.5 Conclusion

This study aimed to establish the presence and profile of microplastics in a stream that is surrounded by suburbs in one of the largest cities in South Africa. The study not only confirmed the presence of microplastics in a stream in Johannesburg but also gave a more detailed look at the ways that stream characteristics may influence the migration of microplastics in the freshwater environment. It was found that microplastics are influenced by aspects such as water depth, flow and obstructions in rivers such as large weirs. The results also show that when establishing the levels of microplastics in a stream, only investigating specific matrices such as water, sediment or an organism that is found in a specific niche, it will not indicate a true reflection of the microplastics in the ecosystem. A snapshot analysis in both abiotic and biotic environments informs future research on aspects to consider when sampling for microplastic contamination in the environment. The hypothesis that microplastics will be found in the Braamfontein Spruit is accepted. The hypotheses that the distribution of microplastics will be affected by stream characteristics and that benthic macroinvertebrates microplastic abundances will have similar trends to microplastic abundances in sediment was found to be plausible. South Africa clearly lacks microplastic research while a large movement in microplastic research is occurring around the globe. The future goal for researchers attempting to fill the research gap of microplastics must be to understand how microplastics behave in the environment to then understand how it would affect the environment. Only then can the true reflection of how microplastics influence the environment be researched and understood, a goal which this study aimed to provide a picture of.

# Chapter 4

# Microplastic distribution of the upper Vaal River

# Chapter 4

## 4.1 Introduction

The Vaal River, the “work-horse” of South Africa, plays a key role in many different economic activities in South Africa, with a combined land catchment area of 196 438 $km^2$ (Wepener et al., 2011; Chokwe and Okonkwo, 2019). Due to various activities such as agriculture, mining, urbanization and reduced capabilities to treat wastewater, extensive research has been completed on the river and organisms in it to determine how the ecosystem has been influenced by these various factors (Retief et al., 2009; Wepener et al., 2011; Malherbe et al., 2016; Rimayi et al., 2016; Hosken, 2018; Plessl et al., 2019; Connell et al., 2020; Moloi et al., 2020). Microplastics seems to be the pollutant which has been under-investigated in the Vaal River system, similarly, around the world, many other freshwater ecosystems were overlooked as more emphasis was placed on the marine environment regarding microplastic research (Blettler et al., 2018; Eerkes-Medrano and Thompson, 2018).

The need for a better understanding of the roll of microplastic research in freshwater environments has therefore led to a recent increase in microplastics research in South African freshwater environments (Verster et al., 2017; Blettler et al., 2018). As previously mentioned, multiple studies investigated the marine environment, examining estuarine fish species and beach sediment (Naidoo et al., 2015; Nel and Froneman, 2015). A paper was then published by Nel et al. (2018), investigating microplastics in sediment and *Chironomus* spp. larvae in the Bloukrans River system, where they found various levels of microplastics in both sediment (160.1 ± 139.5 particles.$kg^{-1}$ dw) and *Chironomus* spp. larvae (1.44 particles.$mg^{-1}$– 5.04 particles.$mg^{-1}$).

Studies by Weideman et al. (2019; 2020) then directly investigated microplastics in both the Vaal and Orange River. Although Weideman et al. (2019) found the presence of microplastics in South African dams, the levels of plastics collected in water were rather low at 0.21 ± 0.27 items. $L^{-1}$. Similarly, it was declared in a second study by Weideman et al. (2020), that microplastics in the water of the Vaal River system (1.7 ± 5.1 items. $L^{-1}$) had limited-long distance transport. A study later published by Dahms

et al. (2020), Chapter 3, on an urban stream in Johannesburg showed that a combination of stream characteristics such as depth and water velocity, could influence microplastic levels in water (705 particles.$m^3$), sediment (166.8 particles.$kg^{-1}$ dry weight) and *Chironomus* spp. larvae (53.4 particles. $g^{-1}$ ww), therefore highlighting the need to research various aspects of a river to better demonstrate the microplastic loads of an environment. These results indicate that a more in-depth analysis of the river must be conducted to determine the health of the ecosystem.

*Clarias gariepinus* is a large benthic fish species found in South Africa (van der Waal and Schoonbee, 1974). They can live for many years and may grow to large sizes, up to 1.7 m in length and 59 kg when fully grown (Skelton, 2001). This species is carnivorous and may feed on a variety of organisms from large and small fish to diatoms and small macroinvertebrates, being adapted to both hunt prey and filter feed (Groenewald, 1963). They don't only play an important part in the environment but have a commercial value through fish farming and is a source of food to rural people (Ali and Jauncey , 2004; Vitule et al., 2006). Due to these factors, this species was deemed to be a good indicator for this study as it can survive in areas with poor water quality, may accumulate high levels of plastic and plays an important social and economic role around the world (Vitule et al., 2006).

In this chapter it was hypothesised that (1) microplastics will be found in the gastrointestinal tract of the benthic fish species *Clarias gariepinus* in the upper Vaal River, (2) a high percentage of the fish will contain secondary microplastics such as filaments and fibres, (3) large dams will accumulate large number of microplastics, (4) river characteristics will influence the distribution of microplastics in the river.

## 4.2 Method

### 4.2.1 Site selection

The Vaal River plays an important role in the South African environment before it joins the greater Orange River system and finally flows into the Atlantic Ocean. In total 4 sites were selected for this investigation on the upper Vaal River (Figure 11). Sites were investigated in pairs, to determine if the large constructions such as the Vaal Dam wall (Figure 18C) and the weir of the Vaal River Barrage (Figure 18D) could

influence microplastic distributions but to also determine a more holistic view of the microplastic loads in the river. Sites were chosen above and below a section of river that passes by the large town Vanderbijlpark (estimated population of 95 840 as of 2011) to assess the influence of the large urban environment on microplastic load (StatsSA, 2011). A site above and shortly below the Vaal Dam wall and the Vaal River Barrage weir were thus used in the study. Sampling was conducted over two weeks from 24 August 2019- 6 September 2019 (two days per site), during the winter season, under low flow conditions similar to the Braamfontein Spruit in Chapter 3, to reduce the influence that external factors could have had on microplastic abundances.

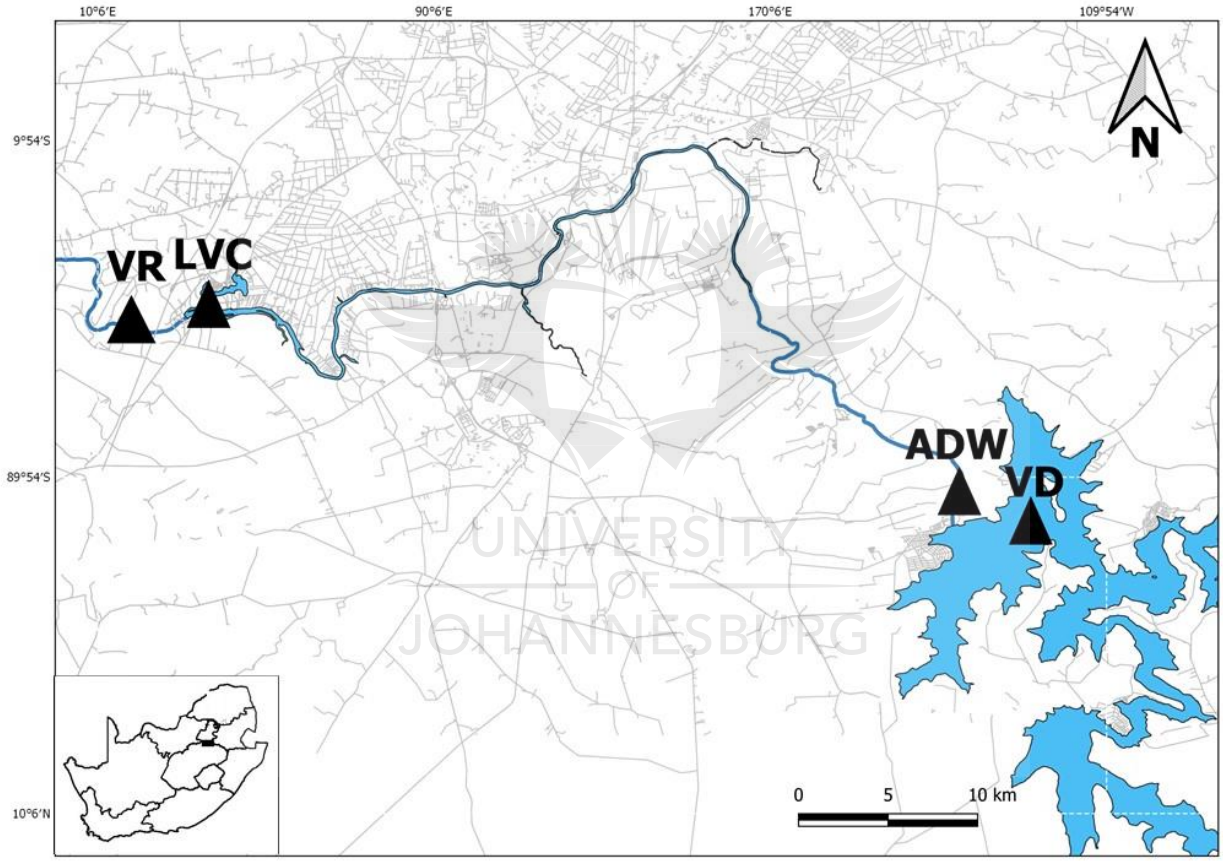


Figure 11: Map of sites investigated in the upper Vaal River. From upstream to downstream: VD- Vaal Dam, ADW- After Dam Wall, LVC- Loch Vaal Club, VR- Vaal Rus.

### 4.2.2 Sample collection

#### 4.2.2.1 *In-situ* water quality parameters

Water quality parameters were measured at each site twice daily (morning and afternoon) coinciding with the collection of water samples. The *in-situ* parameters that were measured included pH, conductivity and Total Dissolved Solids (TDS) in a similar procedure as described by Fischer (2011). The parameters were measured using a handheld Eutech multi-probe water quality meter, after calibration as described in the user manual (Greenfield, 2004). Flow information for the Vaal Dam and Vaal River Barrage on the days of sampling was collected from the "daily flow information 2019" data sheet as reported by Randwater and the Department of Water and Sanitation (Annexures 1 and 2) (Randwater, 2020).

#### 4.2.2.2 Water collection

To determine a better representation for microplastics in the larger river, a greater bulk water sample was collected, compared to the method followed in the smaller Braamfontein Spruit (Chapter 3). At each site a total of 600 L of water was used to determine the microplastic concentration, therefore creating 6 replicates for each site. Therefore, over two days of sampling at each site, 100 L of water was collected three times a day per site (morning, midday and afternoon) to determine a better representation for microplastics at each site. Water collection followed similar procedures to Collicutt et al. (2019). After a thorough washing before each sample collection, a large 25 L container was placed with the lid facing upstream in the water and filled. Caution was taken not to kick up or collect any sediment or materials that may have been trapped in the sediment. The water was then gently poured and filtered through a series of stainless-steel sieves after each sieve was properly washed before each use (4000, 212 and 53 µm). Larger material was removed, and any content caught on the sieves was rinsed, a standardized number of three times and any content collected was saved in small 50 mL containers for laboratory analysis.

### 4.2.2.3 *Clarias gariepinus* collection

After the application and collection of the required permitting (permit number: CPE2-113), *Clarias gariepinus* were caught using a fleet of gill nets (Connell et al., 2020). Nets with the mesh sizes of 70 to 120 mm were used. Nets were laid in the river during the day, parallel to the riverbank, and checked in 2-hour intervals to remove any bycatch safely and to collect any of the target species while minimizing the risk of the fish expelling their gut content. The fish were then weighed, measured and the sexed. The fish were then ethically killed following the procedure as described by the South African National Standards: The care and use of animals for scientific purposes (SANS, 2005). After blunt force trauma to the head, a small incision was made behind the head on the dorsal side of the fish and spinal cord was severed. A small incision was then made from the anus and extended to the gill chamber. Great care was taken not to cut or break any of the intestines during the dissection. The complete gastrointestinal tract was then gently removed and placed in a clear ziplock bag and immediately frozen at -20 °C to prevent any of the gut content from spilling out of the anus or oesophagus and becoming lost. Due to the nature and time of the study, no standardization of the number of a species to use in microplastic research had been established, with some published work using a variety from 3 to 20 individuals of a particular species, 2 to 10 individuals per site or up to 40 to 160 fishes in total (Rochman et al., 2015; Silva- Cavalcanti et al., 2017; Baalkhuyur et al., 2018; Bessa et al., 2018; Kumar et al., 2018; Collicutt et al., 2019; Slootmaekers et al., 2019). Due to time constraints and the availability of the fish species, a total of 39 fish were collected for the study. A total of 6 fish were collected from VD, followed by 9 fish from ADW, 15 from LVC and 9 fish from VR.

### 4.2.2.4 Sediment collection

Sediment was collected at all sites following the collection of water samples to prevent cross-contamination. Approximately 2 kg ww of the top (≈ 10 cm depth) sediment was collected at each site for microplastic extraction and sediment particle size (Nel and Froneman, 2015; Collicutt et al., 2019). The sediment was then placed in ziplock bags (Nel and Froneman, 2015; Collicutt et al., 2019). Challenges were found at LVC with regards to the depth of the environment. In total only, approximately 800 g ww of sediment could be collected there.

### 4.2.3 Laboratory analysis

#### 4.2.3.1 Sediment characteristics

Sediment samples were weighed and dried in an oven at 50 °C to remove any moisture and determine the dry weight. A subsample of the dried sediment was then used to determine the organic content and sediment grain profile. Organic content was determined according to the USEPA (2001) and ASTM (2000) technique. The sediment grain profile was determined by shaking 100 g of dried sediment through a series of stainless-steel sieves (4000 µm, 2000 µm, 500 µm, 212 µm and 53 µm) on a mechanical sediment shaker. After 10 mins the constituents were measured to determine the percentages of each grain size in the specific dimensions named above (Cyrus et al., 2000).

#### 4.2.3.2 Microplastic extraction from water

Water collected were placed in glass beakers and was covered with aluminium foil to prevent air contamination. The volume of the water was measured, and the approximate weight of KOH was measured to create a 10 % KOH solution to remove any organic matter in the water (Gómez-Hernández, 2012). After 24 hours of digestion at room temperature, the remaining content were placed in a covered, clean, glass petri dish for microscopic analysis (Gómez-Hernández, 2012).



#### 4.2.3.3 Microplastic extraction from *Clarias gariepinus*

The whole frozen gastrointestinal tracts of *Clarias gariepinus* were thawed to room temperature, the gut was then weighed. The outer surfaces of the whole gut were then rinsed with distilled water to remove any external contamination and placed in a glass beaker. The intestines were then placed in an oven at 50 °C and left to dry to aid in the digestion process (Collicutt et al., 2019). Three times the volume of the gut of 10 % KOH solution was then added to the intestinal tissue to digest the organic matter but not the plastics that may have been ingested by the animal (Gómez-Hernández, 2012). The beakers were then covered with aluminium foil, placed in the oven and the intestines digested for 5 days at 50 °C in the 10 % KOH solution (Rochman et al., 2015). The digested solution was then placed into 50 mL Falcon tubes and centrifuged

at 3000 rpm for 10 mins (Karami et al., 2016). The supernatant and pellet were then placed in glass Petri dishes after every round of centrifuging for microplastic identification (larger volumes required more rounds of centrifuging to work through the digested solution). Both the supernatant and pellets were investigated to ease counting and prevent the escape of any microplastics that may not have been separated during centrifuging.

### 4.2.3.4 Microplastic extraction from sediment

Sediment was dried for three days at 50 °C to determine the dry weight of the sediment. A subsample of 500 g was then used to determine microplastic contamination through density separation as described by Nel and Froneman (2015), Coppock et al. (2017) and GESAMP (2019). Microplastic density separation remains a key role in the separation of microplastics from sediment, however, some shortfalls may occur (Hidalgo-Ruz et al. 2012; Coppock et al. 2017). Density separation can have high recovery rates (99%) for large microplastics (1-5 mm), although smaller microplastics may have recovery rates of 40-72%. The density of the solution used may similarly influence the recovery rate of certain forms of highly dense plastics. In this study, a hypersaline NaCl (339 $g.L^{-1}$) solution was used for standardization of the method used in Chapter 3 and other studies that have investigated freshwater microplastics in the South African environment such as that used by Nel and Froneman (2015) and Nel et al. (2018) and method development by Coppock et al. (2017). The dried sediment was placed in a glass beaker and filled with the hypersaline solution and filled to approximately 500 mL. The sediment was then stirred vigorously for 2 mins, followed by 18 hours on an orbital shaker which aimed to dislodge any plastics that may have been trapped between the sediment particles or organic matter (Nel et al., 2018; GESAMP, 2019). The sediment was then left to stand for 6 hours to allow sediment to settle and microplastics to remain suspended (Coppock et al. 2017; GESAMP, 2019). The hypersaline solution was then washed through a series of stainless-steel sieves (4000 and 53 µm). The hypersaline solution was then added three times to the sediment, carefully removing any material trapped on the side of the beaker, to increase the likelihood of microplastics being recovered.

### 4.2.3.5 Microplastic identification

Microplastics were identified in a clean, rinsed glass Petri dish using a Carl Zeiss Stemi DV4 dissection microscope. Microplastics had been identified and classified following the colour and shape of the plastics as described in Chapter 2 and 3 (MERI, 2015; Rochman et al. 2015; Windsor et al. 2019). The identification of microplastics followed a step by step guide of elimination, to determine conservative estimates for microplastics, as described by Hidalgo-Ruz et al. (2012) and the Marine and Environmental Research Institute's guide to microplastic identification (MERI, 2015). For an item to be classified as a plastic it had to pass all of the following checkpoints: no organic or cell structure present (fibres and filaments had to undergo further identification through a light microscope), fibres and filaments had to be evenly thick, items that broke apart by gently pressing it with a needle was not counted, if the object had a glass-like texture when pressed with a dissection needle it was excluded, a minimum of one in three items or vastly different items had to pass the "hot needle test" in which a needle is heated up and is gently moved past the item. If the item curled or contracted it was accepted as a plastic. Only if an item had passed all of the previously named checkpoints, was it counted as a microplastic (Gómez-Hernández, 2012; Hidalgo-Ruz et al. 2012; Lusher et al. 2017). Great concern was taken at identifying items of smaller sizes (<0.5mm), as the accuracy of identification decreases drastically at this size. Microplastics were then collected for photomicroscopy and saved for future studies to determine the polymers of plastics that were collected through µ-FT-IR.



### 4.2.4 Contamination control

Contamination control followed similar steps as described by MERI (2015), Coppock et al. (2017) and Lusher et al. (2017). Glassware, containers and all equipment used had been washed with a soap and acid bath system as described by Giesy and Wiener (1977) and was constantly washed before, during and after use with distilled water. Workbenches, microscopes and scales had been washed before use and any containers used had been covered with aluminium foil to prevent airborne contamination. Movement around the workspaces was limited at all time. A black lab coat and purple nitrile gloves were worn during the analyses for the ease of identifying

contamination from clothing. To determine the extent of contamination, negative control samples of distilled water followed the step by step process of the samples from field extraction, drying, digestion, shaking and counting, to determine any possible contamination. If microplastics were detected in the controls, a similar value was removed over the whole group. Water, fish and sediment had unique controls for the various methods each followed. Fish being the most likely to be contaminated during the dissection process had been washed before the drying and digestion process.

### 4.2.5 Statistical analysis

Statistical analysis was performed through the use of IBM SPSS version 26. Microplastic counts were log-transformed to allow the data to be more interpretable. Shapiro Wilk test was conducted to test if the data was normally distributed and a test of homogeneity of variance was conducted to determine if data met the assumptions for further statistical analysis. Pearson's correlations were used to determine correlations between the various microplastic counts. Mann-Whitney U test were then conducted to determine any significant differences between the water microplastics of the various sites. To better understand microplastic loads in fish, the levels in this study were reported from particles per gram of fish ww, particles per gram of gut ww and particles per individual fish. A Spearman's rank correlation test between the various units had shown significant correlations ($p<0.01$) between particles $g^{-1}$ of fish ww, particles $g^{-1}$ gut and particles per fish. Microplastic loads were therefore reported as particles per fish to ease statistical analyses between the various matrices (water, biota, sediment) and to relate to other studies which use the same unit (Annexure 5). Distribution patterns were then visualized by the use of satellite images gathered from Google Earth and a map of the study site was created through QGIS version 3.10.

## 4.3 Results

### 4.3.1 *In-situ* water quality parameters

Between all sites (Figure 12), the pH remained the most constant parameter measured, showing no clear increase or decrease between the sites with a maximum

reading of 9.44 and a minimum of 7.86. In the sites representing the Vaal Dam, the average TDS ranged between 105.5 ppm (VD) and 106.5 ppm (ADW) while the sites representing the Vaal River Barrage downstream revealed increased average readings of 424.5 ppm (LVC) and 545.25 ppm (VR). Conductivity showed a similar trend, (Figure 12), with average readings of 171.65 $\mu S.cm^{-1}$ (VD) and 171.82 $\mu S.cm^{-1}$ (ADW) upstream and average readings of 683.5 $\mu S.cm^{-1}$ (LVC) and 843.25 $\mu S.cm^{-1}$ (VR) downstream. Regarding the discharge of the Vaal Dam and Vaal River Barrage, the Vaal Dam had a discharge of approximately 17.19 $m^3.s^{-1}$ and the Vaal River Barrage an average flow of 10.13 $m^3.s^{-1}$ of water on the days sampling took place after the barriers.

### 4.3.2 Sediment grain profiles

Sediment grain profiles remained similar over the various sites with the exception of ADW. At VD, LVC and VR the particle sizes were dominated by particles predominantly smaller than 212 µm, however, ADW was dominated by particles larger than 212 µm. Following the classification guide (Table 2) of Cyrus et al. (2000), it was determined that over 90% of the sediment profile at VD consisted of medium to very fine sand, with LVC (>65%) and VR (>75%) consisting of similar sediment profiles. ADW consisted primarily (>50%) coarse and very coarse sand with 19% of the sediment consisting of gravel.

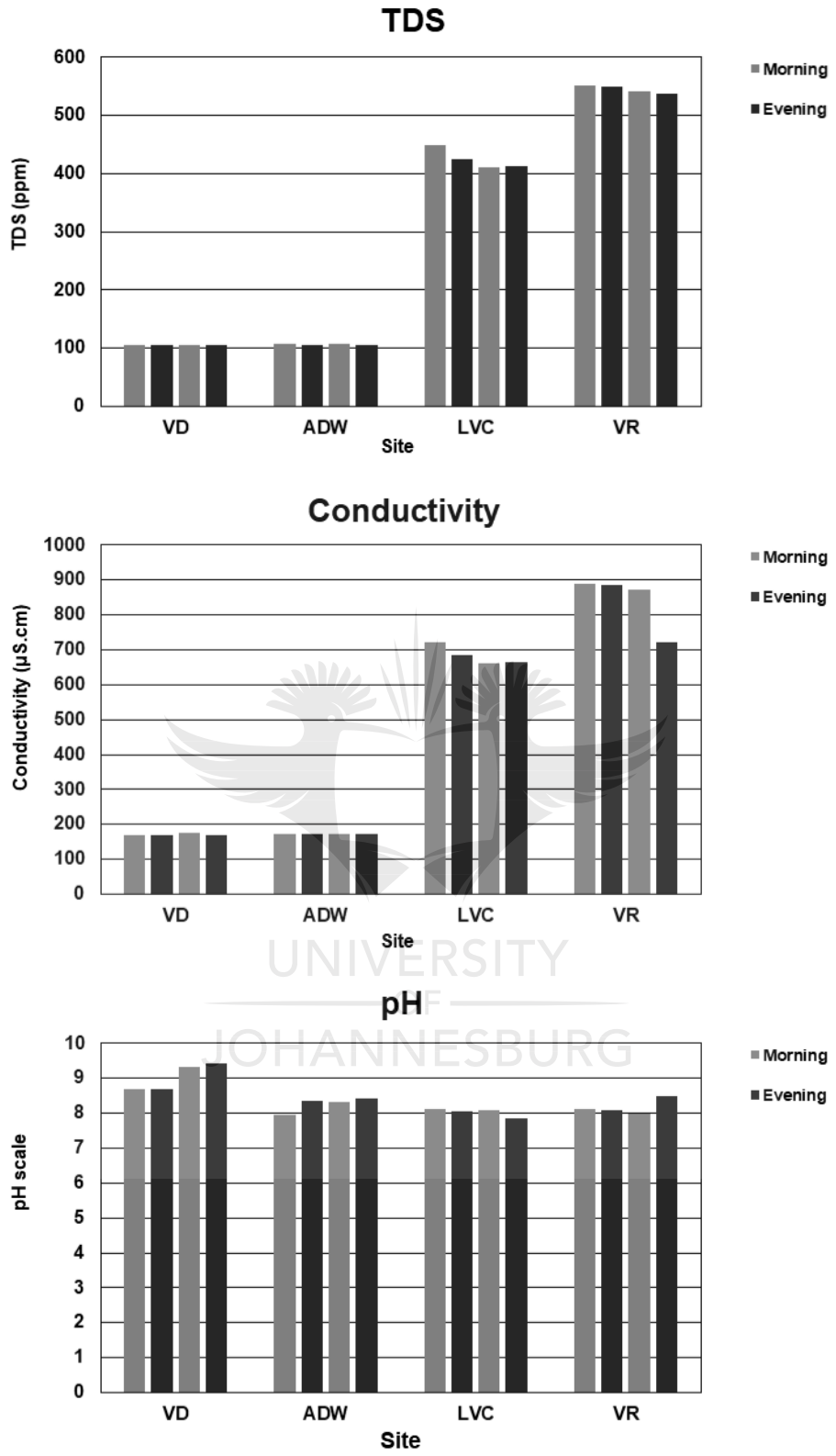


Figure 12: Bar graphs of water quality parameter TDS (ppm), Conductivity (µS.cm$^{-1}$) and pH in the morning and evening on the days of sampling at the site investigated

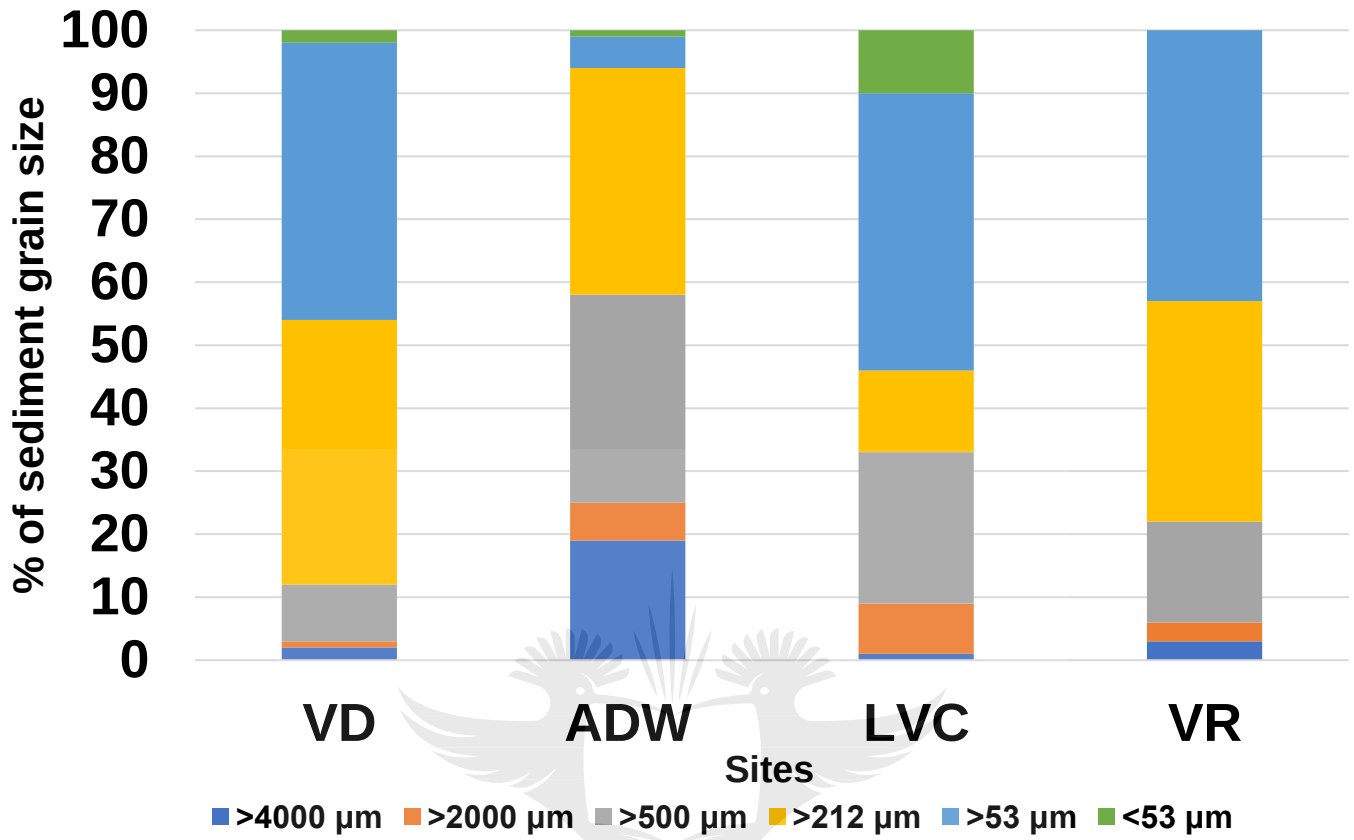




Figure 13: Bar graph indicating the sediment grain profile of the sites investigated in the study indicating the larger sediment particles detected at ADW.

### 4.3.2 Water microplastics

Microplastics were detected in every bulk water sample analysed at every site with a mean of 3299.58 particles $m^{-3}$ through the whole system. The highest concentration of microplastics was detected at ADW with an average of 12398.33 (± 7705.10) particles $m^{-3}$, followed by LVC with an average of 478.33 (± 80,35) particles $m^{-3}$, VR with an average of 236.67 (± 166.21) particles $m^{-3}$ and the lowest readings at VD with an average of 85 (± 52.44) particles $m^{-3}$ (Figure 15A). Mann-Whitney u tests indicated that ADW had microplastic levels that were significantly higher ($p<0.01$) compared to the other sites investigated in this study (Annexure 5). When the significantly high concentration of plastics from ADW is not considered, the average concentration of microplastics found throughout the river is 266.67 particles $m^{-3}$. Overall water samples (Figure 14A) were dominated by angular shaped microplastics (92%), although all these microplastics had been found at ADW. The second most abundant microplastic forms were filaments (8%), followed by other shaped and round microplastics (each <1%). Microplastic colours (Figure 14B) found in the water had a similar pattern being dominated by green (92%) again predominantly from ADW, followed by transparent/white, black and other colours (all contributing approximately 2%), with red being the least abundant (<1%).



### 4.3.3 Microplastic ingestion by *Clarias gariepinus*

Microplastics were detected for the first time in the fish species *Clarias gariepinus* in the Vaal River with 92.3% of all fish tested having at least one microplastic filament and a mean of 7.47 particles per fish. Fish collected in three of the sites had a microplastic prevalence of 100% with ADW only having a 66% prevalence. In total, the abundance of microplastics in fish was highest at LVC (averages of 0.129 particles $g^{-1}$ gut ww, 0.008 particles $g^{-1}$ fish and 16.93 ±8.9 particles per fish). This was followed by VD (0.04 particles $g^{-1}$ gut ww, 0.002 particles $g^{-1}$ fish and 6.67 ± 3.2 particles per fish), VR (0.02 particles $g^{-1}$ gut ww, 0.001 particles $g^{-1}$ fish and 3.4 ±1.4 particles per fish) and ADW (0.073 particles $g^{-1}$ gut ww, 0.002 particles $g^{-1}$ fish and 2.88 ± 1.4 particles per fish). Although ADW had the lowest number of microplastics per fish, Figure 15B, it was the only site where two pieces of large macroparticles were collected in the intestines of two separate fish. One contained what seemed to be a

large piece of fabric and the other a partially degraded condom (Figure 19). The most abundant microplastic shape found in fish were filaments (68%), followed by other shaped (19%), angular (10%) and round (3%) being the least abundant shape as seen in Figure 14C. The colours of the microplastics collected had been primarily dominated by black (29%), transparent/white (27%) and other colours (23%). This was followed by blue (11%), red (6%) and green (4%), the least abundant colours found (Figure 14D).

### 4.3.4 Sediment microplastics

Microplastics had been detected in sediment taken at all sites with an average of 46.7 particles $kg^{-1}$. VR contained the highest abundance of microplastics (68 particles $kg^{-1}$), followed by LVC (53 particles $kg^{-1}$), VD (48 particles $kg^{-1}$) and ADW containing the lowest abundance (18 particles $kg^{-1}$). Microplastics abundances seemed to increase downstream as seen in Figure 15C. Overall the most prevalent microplastic shapes found in sediment were filaments (42%), closely followed by round microplastics (40%) with other shaped objects (14%) and angular (4%) being the least prevalent shapes found. The most prevalent colours found were transparent/white (60%), followed by black (17%), other colours (13%), blue (7%) and green (3%) with no red coloured plastics found as seen in Figure 14E and F.



### 4.3.5 Contamination control

Control samples containing distilled water were prepared concurrently with all samples during preparation, extraction and counting, to determine any possible contamination from the researcher. The total plastics found were subtracted over all samples to determine the influence of contamination over the whole dataset. The control for water contained 6 filaments (4 black, 1 blue and 1 transparent/white) found from the various samples taken. No contamination was detected in sediment and fish samples.

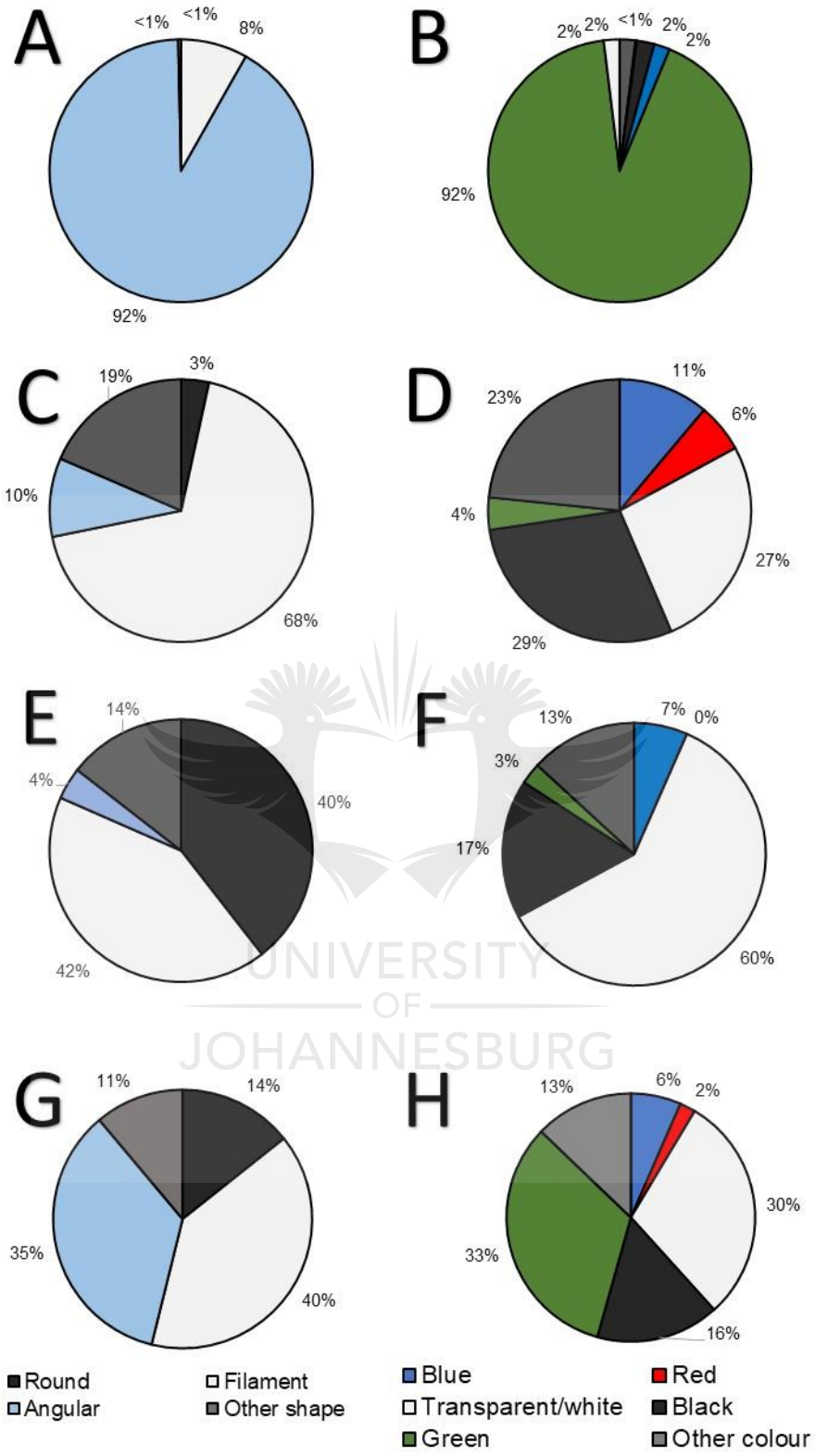


Figure 14: Pie charts illustrating the different forms and colours of microplastics collected in A (forms in water), B (colours in water), C (forms in *Clarias* gariepinus.), D (colours in *Clarias* gariepinus), E (forms in sediment), F (colour in sediment), G (all forms in total) and H (all colours in total) in the upper Vaal River.

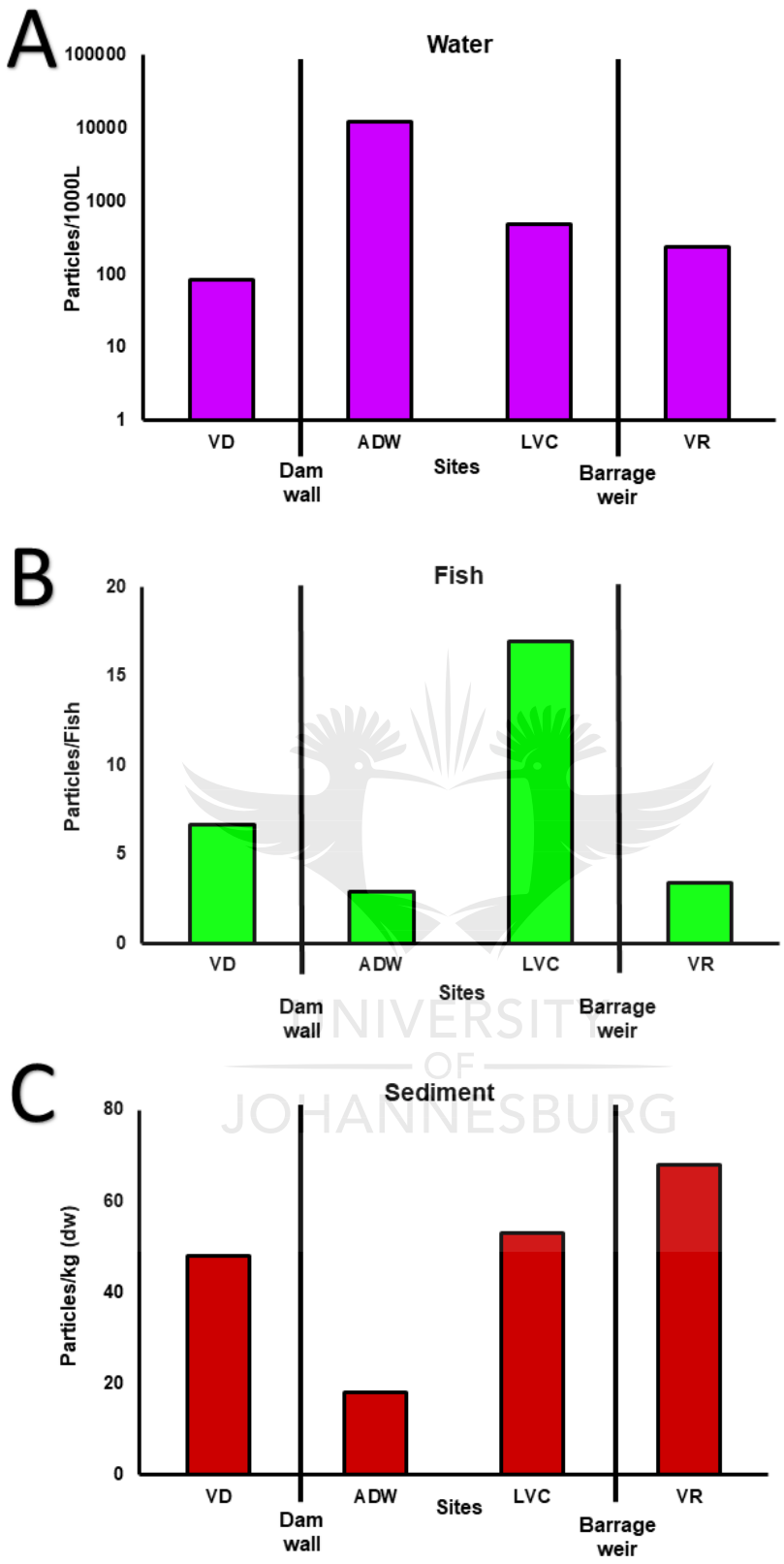


Figure 15: Bar graphs of the mean total number of microplastics found in water (A logarithmic scale), fish (B) and sediment (C). dw – dry weight

## 4.4 Discussion

Microplastics have now been detected in both the water column of the upper Vaal River system, the sediment and the apex predator fish species, the African sharptooth catfish, *Clarias gariepinus* (Weideman et al., 2019, 2020). The results of this study provide an interesting distribution of microplastics through the ecosystem from four distinct sections of the river. Overall microplastic research in the global environment is dominated by large amounts of microfibres and filaments detected in various studies from marine and freshwater sources (Rochman et al., 2015; Li et al., 2018; Nel et al., 2018; Mcllwraith et al. 2019; Weideman et al., 2019). In this study, the shape that was most commonly found was filaments and green angular shaped microplastics, of which the latter dominated in the water column of ADW (Figure 14A).

The Vaal River remains as an interesting focal point in microplastic studies. It has been found both in this study and by the studies of Weideman et al. (2019; 2020), that the microplastic concentrations in the Vaal Dam remained low. However, to compare the levels of microplastic loads in different environments and studies remain challenging without a standard method to investigate microplastic concentrations (Gómez-Hernández, 2012; Hidalgo-Ruz et al. 2012; Lusher et al. 2017). Reporting microplastic levels in only the water of various environments may only present a fraction of the true perspective of the overall plastic pollution of a freshwater environment, similarly, not providing environmental parameters on the time of sampling may also influence the reliability and context of the microplastic abundances that were found.

The microplastic distribution patterns in water, sediment and fish in this study present a more holistic view of how to determine and report realistic microplastic levels in an ecosystem (Figure 16A). The water microplastics behaved similarly to that of the smaller order streams, the Braamfontein Spruit assessed in Chapter 3, as well as other smaller order streams of the Bloukrans River in South Africa with a massive increase of microplastics in ADW (Nel et al., 2018; Dahms et al., 2020). This is attributed to a high increase in flow occurred, compared to the other sites where streamflow was almost undetectable, such as in the Vaal Dam and Vaal River Barrage (Randwater, 2019). This indicates the unique relationship between how microplastics behave in the water which was found in both the Braamfontein Spruit and the upper Vaal River. The Vaal Dam wall (Figure 18CG) is the major obstacle dividing VD from ADW and may

have shown some influence on the microplastic loads as it directly influences the movement of water that enter or exit the dam. In VD, the microplastic load in the water was the lowest compared to the other sites, something that was expected with regards to how the still-standing water may allow more microplastics to gain a layer of biofilm, increase in density and may sink to the sediment layer (Guo and Wang, 2019, Watkins et al., 2019). Here the microplastics may then increase in abundance in the sediment.

In a research article by Watkins et al. (2019), the researchers highlighted how significant differences were found between water samples as well as sediment microplastic loads in the reservoirs of the dams compared to the sites leading up to and exiting the dam. These similar trends were detected in VD, Figure 16 VD, where 85 particles (± 52.44) $m^{-3}$ was detected in the water column but 48 particles $kg^{-1}$ was detected in the sediment, a ratio of 1 particle $L^{-1}$ of water to 48 particles $kg^{-1}$ of sediment with large differences found after the dam. The vast difference between water and sediment microplastics was not detected in ADW, where the ratio of plastic in water was only marginally lower to sediment (1 particle $L^{-1}$ of water to 1.45 particles $kg^{-1}$ of sediment). The closer relationship at ADW may be due to the high microplastic load in the water, which was significantly higher ($p<0.01$) compared to the other sites that were investigated.

The higher levels located at ADW could be due to the great depth at the site (>2m) and the flow of water constantly exiting the Vaal Dam wall (17.19 $m^3.s^{-1}$ at the time of sampling) (Randwater, 2019). When the mean microplastic loads found in the water of VD (85 particles $m^{-3}$) is taken into account with the flow of water exiting the dam (17.19 $m^3.s^{-1}$), (Figure 17ADW), the dam wall could be releasing approximately an average of 1461.15 particles every second directly into the rapidly flowing water in the river after the dam wall. Similarly, if only half of the microplastic concentration that was detected in VD was used, it would equate to 730 particles released into that section of river after the dam wall every second. The microplastics expelled here would then have to travel 1.5 km downstream, past a bridge and series of rapids to the site ADW where sampling took place (Figure 17ADW). In this section of the river a large weir is located downstream, which allows for an increase in water volume here. Similarly, the topography of the river changes, with a much wider cross section, allowing it to gather higher amounts of water and plastic being expelled, which may explain the concentration of microplastics found in the bulk water samples at ADW of 12398.33

(±7705.1) particles $m^{-3}$). The section of river at ADW therefore creates a bottleneck, which gathers high volumes of water and therefor plastics, as the results indicate.

The Vaal River Barrage can be considered the next major location which divides LVC and ADW with the large weir located at the Vaal River Barrage (Figure 18D). The weir found between LVC and VR functions differently from the Vaal Dam wall which divides VD and ADW (Figure 18C). The Vaal Dam wall is a large, high barrier, which increases water volume with excess water passing over the dam wall gates, with a spillway releasing a constant amount of water under the dam wall. The Vaal River Barrage weir, however, is a much shorter structure, which regulates and diverges water flow through a series of gates unlike the dam wall. The barrage weir would allow plastics to settle out as previously discussed (Figure 9) and again the results indicate this with a rapid increase in sediment microplastics in LVC compared to both VD and ADW with a ratio of 1 particle $L^{-1}$ of water to 110.8 particles $kg^{-1}$ in sediment (Figure 17LVC). The flow of water moving through the Vaal River Barrage between LVC and VR is lower than that of the Vaal Dam wall between VD and ADW (Randwater, 2020). Therefore, the water microplastic concentration in VR decreased slightly from LVC, as the sediment concentration increased, indicating that the microplastics may be settling out in the dry season (Figure 17 VR). The method that the Vaal River Barrage weir functions could therefore indicate why a similar spike in water microplastic abundances did not occur, as was seen after the Vaal Dam wall.

The water quality at LVC and VR was also clearly reduced from the upstream sites as seen in Figure 12. With regard to the water quality, the conductivity and TDS readings at LVC and VR were much higher than the upstream sites. VR seemed to have the lowest water quality as the TDS reading was higher than the prescribed safe level for drinking water by the Environmental Protection Agency (EPA), indicating more pollution having entered the river, which could lead to higher overall microplastic abundances (Gatidou et al. 2018; Li et al. 2018; Sun et al. 2019; Wang et al. 2019; EPA, 2020).

The pattern found throughout the system was then investigated statistically through a Pearson Correlation of the log-transformed means, to identify the possible relationship between water, fish and sediment microplastic loads through the system. The tests indicated an inverse Pearson' *r* correlation of -0,883 between sediment and water microplastic loads, the relationship however was not significant, ($p<0.117$). The

relationship between microplastic loads in biota and their habitat must be investigated in further microplastic studies. It is key to the future of microplastic research in ecotoxicology and toxicological research of microplastics to understand how microplastics behave in the specific niches where animals are found, to accurately determine and understand their effects.

Investigating the microplastic loads of biota remains key to microplastic studies and must be continued in future studies. The results from microplastics found in *Clarias gariepinus* in the Vaal River support this statement. Reporting microplastic levels in animals remains troublesome and can be reported in various ways for different animals (Boerger et al., 2010; Rochman et al., 2015; Guzzetti et al., 2018; Wang et al., 2019; Dahms et al., 2020).

The microplastic levels in the fish ranged between the sites from 2.88 to 16.93 particles per fish with a mean of 7.47 particles per fish. These levels were much higher than that found in the smaller benthic fish species *H. littoralle* (3.6 particles per fish) by Silva-Cavalcanti et al. (2017), juvenile chinook salmon (1.15 ± 1.41 particles per fish) by Collicutt et al. (2019) and in several fish species (0.2 ± 0.5 particles per fish) from a freshwater lake in Germany by Roch et al. (2019). This indicates how a larger predatory benthic fish species such as *Clarias gariepinus* may be ingesting a much larger number of microplastics as it feeds on a wider variety of organisms from benthic macroinvertebrates, small to large fish and birds and has the ability to filter feed on surrounding microalgae (Groenewald, 1963). In comparison, *Clarias gariepinus* had on average more microplastics than that of several marine fishes investigated by Rochman et al. (2015) (0 - 2.5 particles per fish) with only the 7 individuals of the family Carangidae (5.9 ± 5.1 particles per fish) and 17 fish of the species *Decapterus macrosoma* (2.5 ± 6.3 particles per fish) having similar levels of microplastics.

The high loads of plastics found in *Clarias gariepinus* provide a worrisome indication of the health of the species, as a biomarkers study on juvenile *Clarias gariepinus* indicated that plastics lead to a variety of biomarker responses, but more research on the plastics with other environmental toxins are of the utmost importance to understand how this species is influenced (Karami et al., 2016; Barboza et al., 2018). In a recent toxicological study by Barboza et al. (2018), the researchers determined that a mixture of microplastics with toxins such as Hg create a more toxic environment, which could prevent the fish species *Dicentrarchus labrax* from excreting the toxins

through their gills. This led to the bioconcentration of the Hg in their gills and allowed it to bioaccumulate in the liver of the juvenile fish. *Clarias gariepinus* does not only play an important ecological role in South African ecosystems but is an important food source for people living close to the river (Ali and Jauncey, 2004). The health of people could similarly be under threat as more studies are revealing microplastic ingestion by humans (Peixoto et al., 2019).

Fish microplastic abundances varied similarly to that of water and sediment between the sites. In VD the fish had approximately 6.67 particles per fish, the second highest of the sites tested. The number of plastics then rapidly decreased in ADW to 2.88 particles per fish before it then rapidly increased to 16.93 particles per fish in LVC. This site was expected to contain the most microplastics in fish with the increased pollution in this area, which can be seen in the reduction of water quality in the stretch of the river as seen in Figure 12 and has been highlighted previously in a study by Wepener et al. 2011). The level of microplastics per fish then decreased further downstream at VR to 3.4 particles per fish. When compared to the water and sediment patterns, initially it seemed to follow a similar pattern to the sediment microplastic distribution pattern from VD to LVC, although at VR it followed a pattern similar to that of water. Statistical analysis could however not concretely establish a relationship between microplastics in fish with water and sediment.

Furthermore, similar to the Braamfontein Spruit in Chapter 3, the sites (VD, LVC and VR) with a clearly finer sediment profile of fine sand had trapped more microplastics, whereas ADW with its larger coarse and gravel sediment particles, could trap less and release higher microplastics loads into the water column as indicated in the high microplastic counts in the water microplastics samples of ADW.

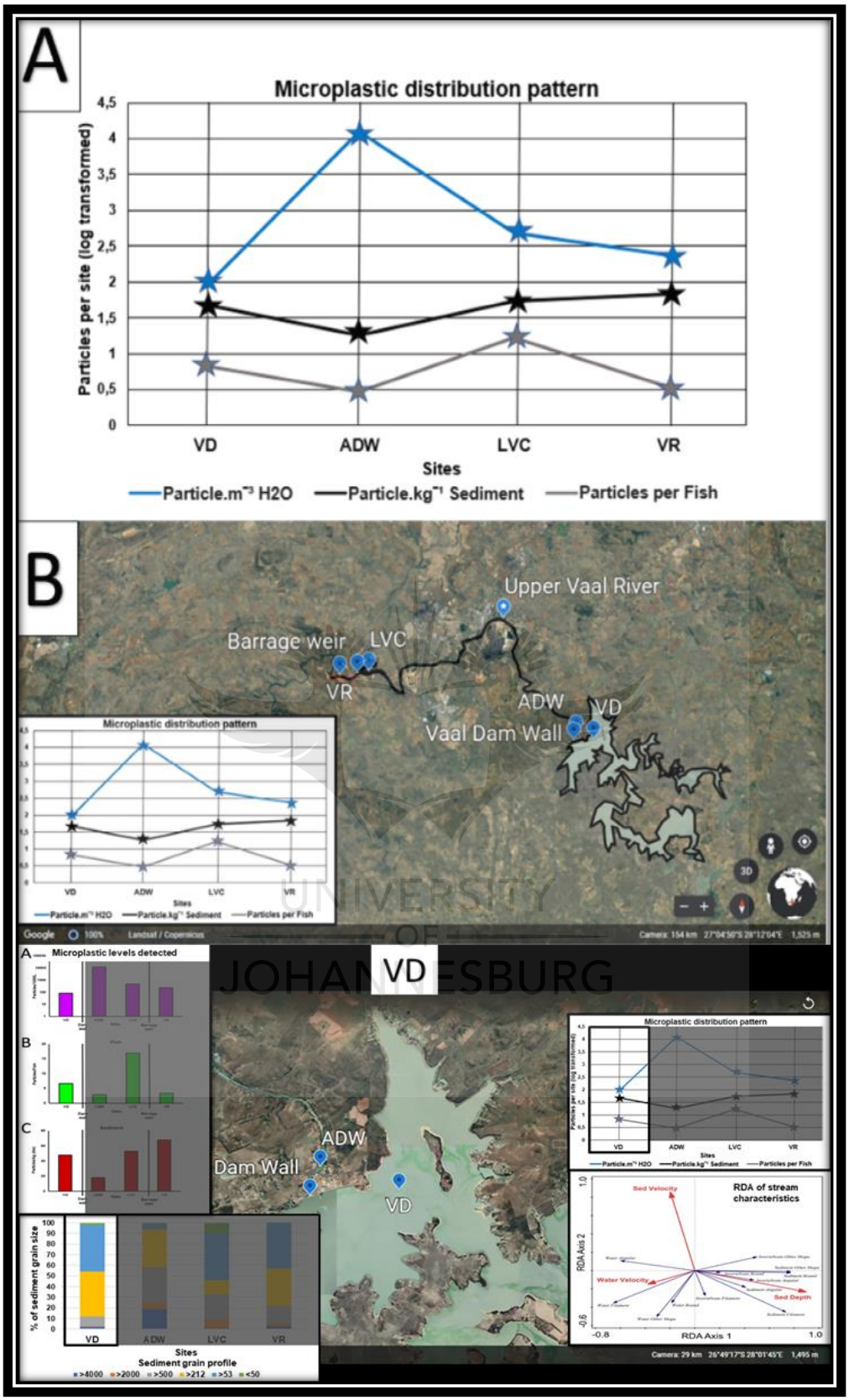


Figure 16: Distribution maps of microplastics in A [water (blue), sediment (black) and *Clarias gariepinus* (grey)]. B (Satelite image of microplastic distribution in the upper Vaal River). VD [Microplastic levels (top left) and characteristics (sediment grain profile-bottom left), that may have influenced its distribution in Vaal Dam with the RDA of Chapter 3 as a reference to the effect of environment parameters (bottom right)] (Dahms et al., 2020; Google Earth, 2020).

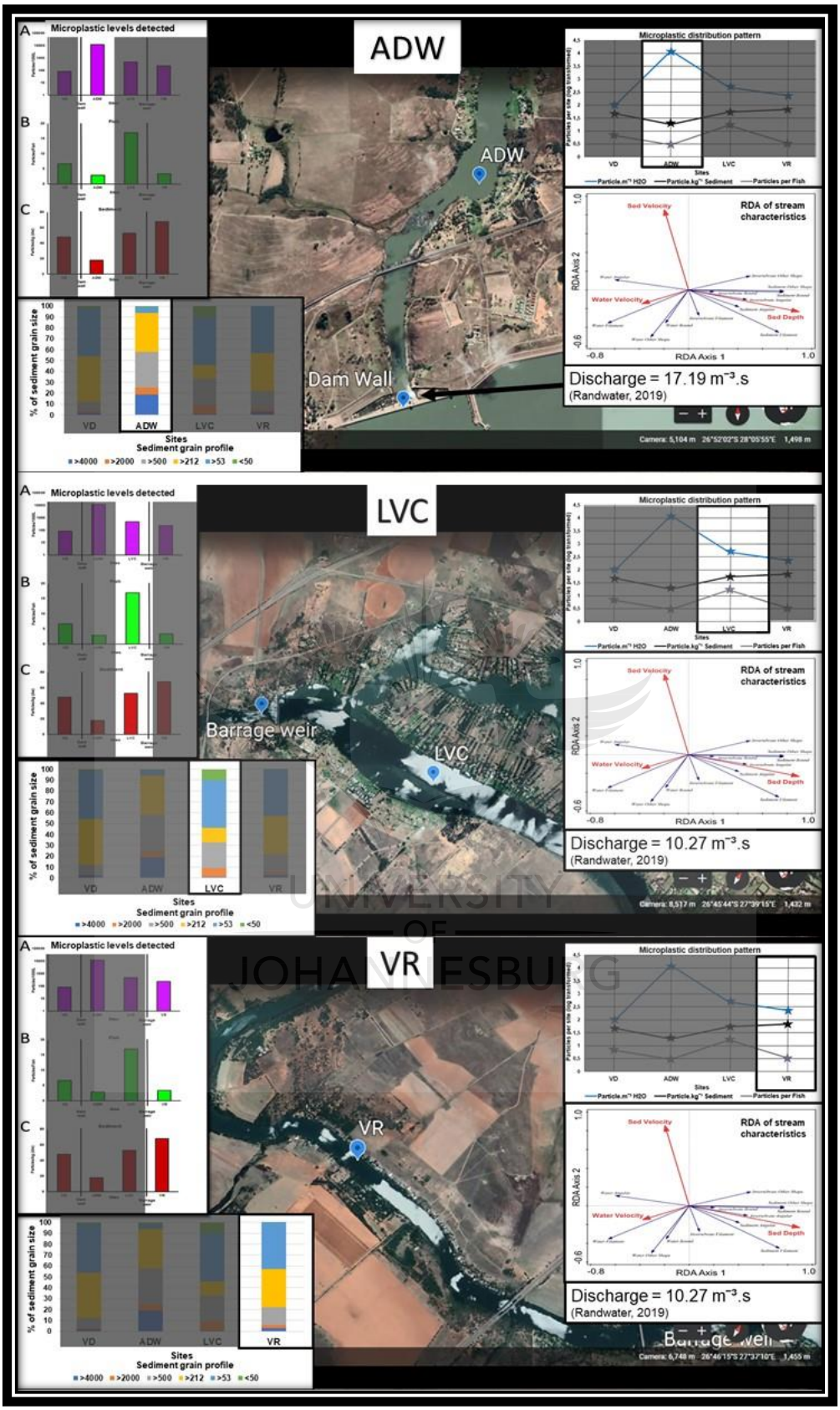


Figure 17: Microplastic distributions at ADW, LVC and VR with river charateristics that may have influenced its distribution. Microplastic levels (top left), Sediment grain size (bottom left), distribution (top right) and the RDA of Chapter 3 as a reference to the effect of environment parameters (bottom right) (Dahms et al., 2020; Google Earth, 2020; Randwater, 2020).

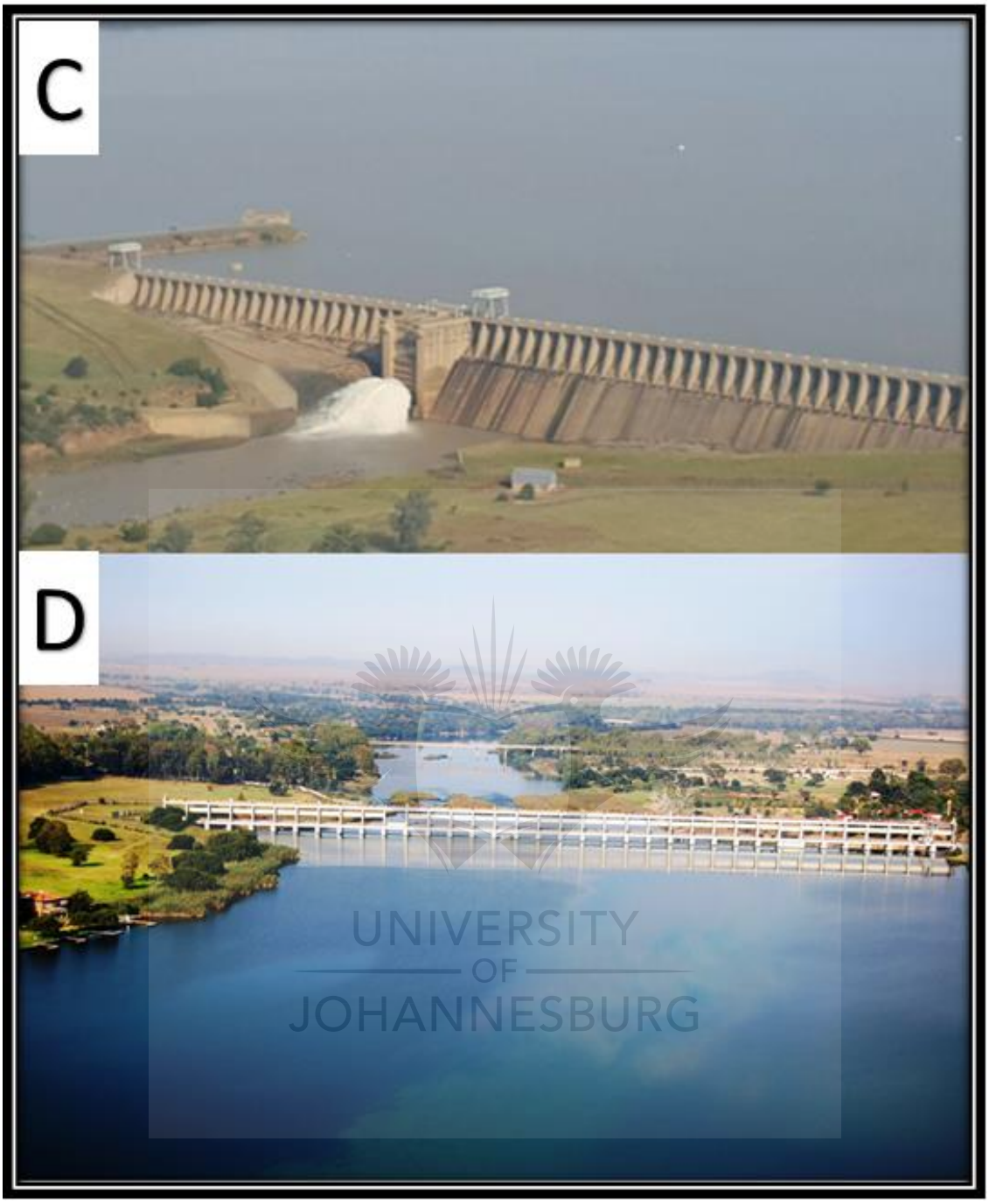



Figure 18: Major obstruction that may have influenced microplastic distributions. C (Vaal Dam wall found between VD and ADW). D (Vaal River Barrage weir located between LVC and VR) (du Toit, 2016; Vaal explorer, 2019).


Figure 19: Photo of a degraded condom (possible macroplastic) collected in the gastrointestinal tract of a fish collected in this study at ADW.

## 4.5 Conclusion

This study provides evidence of how microplastics have entered various parts of the ecosystem of the upper Vaal River. It also provides the first indication of microplastics in the fish species *Clarias gariepinus*, a top predatory fish and economically important species in South Africa. The microplastics found in the fish remain high compared to similar studies of benthic freshwater fish, highlighting the need for further research on how the species may be influenced by microplastics. The results found in this study similarly highlights the difficulty to understand and determine this, as the various environments contain different microplastic abundances and therefore determining the right concentrations to use in toxicological studies remain troublesome. More research must be completed on relating an organism to its environment and therefore the probability that it might be exposed to certain levels of microplastics. The hypotheses that (1) microplastics will be found in *Clarias gariepinus* and that (2) it would be a higher percentage of secondary microplastics are accepted. The hypotheses regarding (3) the ability for dams to act as a sink is plausible to some extent, more research is required. The large dam was able to hold more plastics in the sediment, as it could settle down, but the constant release of water forces a high and steady flow of plastics downstream. Although (4) water and sediment showed an inverse relationship, fish could not be related to the two matrices but seemed to relate more to sediment than water. Microplastic research remains key, with the following step to determine the microplastic polymers found as well as the toxins that may have been absorbed to the plastic particles from the environment.

# Chapter 5

# General discussion

# Chapter 5

## 5.1 Microplastics as a part of the environment

In recent years, researchers have investigated a variety of environments to determine the overall spread of microplastics from freshwater and marine environments to soil, biota and in the atmosphere all over the world (Li et al., 2018; Chen et al., 2020; Pirsaheb et al., 2020; Yao et al., 2020). The distribution of microplastics is so extensive it can almost be presumed that microplastics will be found in any environment investigated (Rochman et al. 2015; Horton et al., 2017; Blettler et al. 2018; Eerkes-Medrano and Thompson, 2018; Li et al. 2018; Peng et al. 2018; Geilfus et al., 2019). The characteristics of microplastics allow it to be transported not only by ocean and river currents but easily through the atmosphere suggests that areas with little to no plastic litter can be polluted with microplastics (Iwasaki et al., 2017; Yao et al., 2020). This can be seen where the presence of microplastics have been found in protected and isolated environments such as in the mountain Lake Hovsgol in Mongolia, the seas around Antarctica and in protected areas in the United States, where microplastics had been deposited through rain droplets (Li et al., 2018; Augusto et al., 2020; Brahney et al., 2020). This describes a world that may be covered in microplastics soon if it is not already so.

The spread of microplastics in the environment must be critically investigated, to understand the plastic cycle completely (Blettler et al., 2018). The pathways that were found in this study and similarly in studies around the world, provide an indication of how plastics may be transported by organisms and by the environment itself (Li et al., 2018; Dahms et al., 2020). After microplastics enter the environment, it can be transported through various means (Li et al., 2018). Plastics can be recycled or may end up in large waste sites (Turner, 2019). Here the plastics, primarily from e-waste, are in contact with metals such as lead, cadmium and bromine, which have been detected on marine litter found on beaches of the United Kingdom (Massos and Turner, 2017, Turner, 2019). The plastics may then enter the environment through various means, from pure waste, the washing of clothes that release microfibers in greywater, road dust or spills of nurdles and other plastic material from shipping plastic around the world (Massos and Turner, 2017; Li et al., 2018; McIlwraith et al., 2019;

Rezaei et al., 2019; Dahms et al., 2020; Kitahara and Nakata, 2020; Tunnel et al., 2020; Yukioka et al., 2020). These plastics then form part of the various ecosystems in which they are found. They can then be ingested by organisms such as *Clarias gariepinus*, a source of food to humans, with no concrete evidence whether these harmful chemicals absorbed by the plastic can be transferred to the fish and then the humans after being consumed.

The rivers may then act as a highway, transporting microplastics to the various oceans where they can deposit their microplastic loads in oceans around the world, however, it is highly probable that the plastics in streams and rivers may become trapped in the ecosystem in sediment, plant growth such as algae or become ingested by organisms (Nel et al., 2018; Näkki et al 2019; Dahms et al., 2020; Feng et al., 2020; Weideman et al., 2020). When microplastics enter a stream in an urban setting, low density plastics may be transported in the surface water downstream to the rivers (Weideman et al., 2020). However, streams and rivers are complex systems, with areas of different flow velocities, depths, sediment, blockages from vegetation, manmade structures and seasonal variations which can influence plastics in all these systems seasonally (Nel et al., 2018; Dahms et al., 2020; Feng et al., 2020). Areas of increased flow show higher microplastics in water, as the turbulent water removes microplastics from the sediment and washes it downstream as seen in both the Braamfontein Spruit and Vaal River, with similar evidence seen in the Bloukrans River with seasonal variation, urban streams of New Zealand and the difference of microplastic levels leading up to, in and after dams (Nel et al., 2018; Dikareva and Simon, 2019; Watkins et al., 2019; Dahms et al., 2020)

When it finally enters the oceans, currents can transport large quantities of plastic waste (Kane et al., 2020). Areas with stronger currents can transport higher numbers of plastics, where it can collect in levels up to $8.3 \times 10^{-6}$ $m^{-3}$ which have been detected in the North Pacific Ocean (Iwasaki et al., 2017; Brandon et al., 2019; Kane et al., 2020). This allows plastics to be caught in major ocean currents which transport them around the globe. As the plastics increase in density, it may then settle the deepest ocean environments or may become ingested by various animals (Rochman et al., 2015; Peng et al., 2019).

The results of this study and several others described an almost migratory pattern for microplastics. The plastics display a complex migratory pattern which leads to a variety

of outcomes instead of merely flowing downstream and ending up in the oceans. The question that then begs to be answered is whether microplastics can be transported from the oceans into the atmosphere by sea spray, waterspouts and being ingested by migratory sea birds. This would complete a cycle of microplastics from oceans, to air, land, rivers and finally the oceans again.

## 5.2 Dangers of microplastics in the environment

As microplastics become trapped in the ecosystems for long periods they interact with the surrounding environment on a physical and chemical level (Guo and Wang, 2019). As previously described, microplastics go through several changes when it has been in the environment for an extended period, from density changes, surface cracks, decrease in size and colour changes (Guo and Wang, 2019). These extended periods allow microplastics in the environment to act as sponges, allowing metals to bind to the surface (Guo and Wang, 2019). Studies by Ashton et al. (2010) and Massos and Turner (2017) found interesting relationships between microplastics and metals in the environment. Ashton et al. (2010) determined metal concentrations of major metals (Mn, Al and Fe) as well as several trace elements from beached plastics. They determined that plastics could be used as a method for determining metal concentrations in the environment and that further research was key (Ashton et al., 2010). Research now further indicates that plastics suspended in the sea may accumulate higher levels of metals than originally found (Turner, 2016; Massos and Turner, 2017). A study by Turner (2016), high concentrations of Pb were found in foam and plastics up to 17 500 µg $g^{1}$ that were collected on several beaches. The study reflects the need for further research not only on metals on plastic litter but the chemicals that are used in plastic production, such as flame retardants, which relate to high levels of Cl and Br found on beached plastics by Turner (2016).

The high concentrations of metals and toxins in the plastic must also be accredited to the additives used in the production process (Hahladakis et al., 2018). Originally with the production of plastics, new polymers of plastic were developed with different qualities from density, durability and would be used in different ways (Turner, 2019). It was more affordable later on to include various additives that would alter its characteristics (Turner, 2019). This would prove to be a Pandora ’s Box scenario for

plastics in the environment as these additives would not only be able to leach into the environment but play a larger role in the recycling of plastics (Hahladakis et al., 2018; Turner, 2019). These additives once used, would always remain in the plastic, meaning toxins such as the heavy metals Cd, Pb and similarly harmful chemicals such as Polybrominated Diphenyl Ether (PBDE) and triclosan, would remain in plastic, even when recycled (Hahladakis et al., 2018; Turner, 2019). The issue with recycling is broadened as the infrared scanning used to determine plastic doesn't function with black plastic, which is therefore not recycled (Turner, 2019). Black plastic poses the danger that it was most likely recycled from electronic waste which was not scanned or separated accurately (Turner, 2019). This may explain the high levels of Pb found on black plastics collected by Massos and Turner (2017).

Once these plastics with high concentrations of harmful substances become ingested by an organism, the harmful chemicals can release and be absorbed by the animal (Li et al., 2018; Guo and Wang, 2019). With more and more animals being discovered with plastic litter and similar material in their gut and high levels of plastic in their surrounding environment, research must be conducted with plastic that has either been left in the environment to absorb toxins or from plastic litter collected in the environment.

# Chapter 6

# Conclusion

# Chapter 6

## 6.1 Conclusion

This study has not only investigated freshwater streams in rivers in Africa for microplastics but has determined how various aspects of the environment influence its spread. Once the transportation of plastics in the environments is understood and the relationships found, the effects of plastics on animals will be better understood. Comparative research between microplastics in the environment and the animals found there is key to understanding the danger that these plastics pose.

This study determined that microplastics are found in lower and higher-order streams in South Africa and the relationships between the transport of these plastics from small order streams to large rivers must be investigated further. Environmental characteristics from both the river, riparian vegetation, surrounding human population and the atmosphere must be taken into consideration as it may influence the spread of these plastics. If enough data can be gathered and relationships understood, maybe a model can be created to determine the movement of plastics.

For this to occur, scientists must determine the correct way forward to assess microplastics in the environment and whether various methods are used have similar results. What remains key is that a system of microplastic analyses is developed which remains quick, cheap and requires little training, to increase microplastic research, which is drastically needed in various environments.

A trend was found in the different environments of both the Braamfontein Spruit and upper Vaal River, where sediment tended to retain higher concentrations of plastics than water. Benthic organisms are most likely at higher risk of the surrounding microplastics. Both studies found high levels of plastics in benthic macroinvertebrates and fish. These two organisms are at opposite ends of the food chain in South African rivers, suggesting the presence of microplastics throughout the food chain is highly possible.

The study recommends that microplastic research is required through all environments with new and creative investigations, looking at various species of animals from both the environment and their exposures to microplastics in laboratory experiments. The

dangers and lack of research of these pollutants must be communicated to the greater population, the government of South Africa and the world as a stern warning to the unseen dangers that are being found around the world.

Multiple aims and objectives were set in this study. The study can therefore conclude that, as hypothesised, microplastics were found in the ecosystems of both the Braamfontein Spruit and upper Vaal River. The microplastics can were detected in the biota within the Braamfontein Spruit (*Chironomid* sp.) and upper Vaal River (*Clarias gariepinus*). In both investigated sites, filaments were the most widespread microplastic and should therefore require the greatest research in future studies.

Most importantly, the results from both waterbodies show how microplastic distribution could be influenced by the stream characteristics itself, as hypothesized in Chapter 4. These include structures that may increase or decrease flow of rivers or streams, the profile of the sediment, the depth and structure of the river itself, surrounding anthropogenic activities and the surrounding atmosphere. All hypotheses in the study were therefore accepted, except the hypothesis that benthic macroinvertebrates indicate similar trends in microplastic distribution to sediment, which could not clearly be established. By accepting the hypotheses in both Chapter 3 and Chapter 4, the aims and objectives presented in Chapter 1 have been achieved and has led to a better understanding of the state of microplastics in freshwater environments.

# Chapter 7

# References

# Chapter 7

## References


Akdogan, Z., Guven, B., 2019. Microplastics in the environment : A critical review of current understanding and identification of future research needs. Environ. Pollut. 254, 113011. https://doi.org/10.1016/j.envpol.2019.113011

Akindele, E.O., Ehler, S.M., Koop, J.H.E., 2019. First empirical study of freshwater microplastics in West Africa using gastropods from Nigeria as bioindicators. Limnologica. 78, 125708. https://doi.org/10.1016/j.limno.2019.125708

Ali, M.Z., Jauncey, K., 2004. Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell 1822). Aquac. Int. 12, 169–180. https://doi.org/10.1023/B:AQUI.0000032065.28059.5b

Anderson, J.C., Park, B.J., Palace, V.P., 2016. Microplastics in aquatic environments: Implications for Canadian ecosystems. Environ. Pollut. 218, 269–280. https://doi.org/10.1016/j.envpol.2016.06.074

Arthur, C., Baker, J. and Bamford, H., 2008. International research workshop on the occurrence, effects, and fate of microplastic marine debris. In *Conference Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. Sept*. 9-11.

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014

ASTM, 2000. American Society for Testing and Materials Standards on Environmental Sampling. Standard practice for preparation of sediment samples for chemical analysis. Conshohocken, PA, USA. 163–165.

Augusto, A., Tomio, Y., Rosso, B., Gambaro, A., Sfriso, A., Corami, F., Rastelli, E., Corinaldesi, C., Mistri, M., Munari, C., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay Ross. Environ. Int. 137, 105587. https://doi.org/10.1016/j.envint.2020.105587

Baalkhuyur, F.M., Bin Dohaish, E.J.A., Elhalwagy, M.E.A., Alikunhi, N.M., AlSuwailem, A.M., Røstad, A., Coker, D.J., Berumen, M.L., Duarte, C.M., 2018. Microplastic in the gastrointestinal tract of fishes along the Saudi Arabian Red Sea coast. Mar. Pollut. Bull. 131, 407–415. https://doi.org/10.1016/j.marpolbul.2018.04.040

Barboza, L.G.A., Vieira, L.R., Branco, V., Carvalho, C., Guilhermino, L., 2018. Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in *Dicentrarchus labrax* juveniles. Sci. Rep. 8, 1–9. https://doi.org/10.1038/s41598-018-34125-z

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044

Biginagwa, F.J., Mayoma, B.S., Shashoua, Y., Syberg, K., Khan, F.R., 2016. First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia. J. Great Lakes Res. 42, 146–149. https://doi.org/https://doi.org/10.1016/j.jglr.2015.10.012

Blettler, M.C.M., Abrial, E., Khan, F.R., Sivri, N., Espinola, L.A., 2018. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. Water Res. 143, 416–424. https://doi.org/10.1016/j.watres.2018.06.015

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Brahney, J., Hallerud, M., Heim, E., Hahnenberger, M., Sukumaran, S., 2020. Plastic rain in protected areas of the United States. J. Sci. 368, 1257-1260. https://doi.org/10.1126/science.aaz5819

Brandon, J.A., Freibott, A., Sala, L.M., 2020. Special issue-letter: Patterns of suspended and salp-ingested microplastic debris in the North Pacific investigated with epifluorescence microscopy. Limnol. Oceanogr. 5, 46–53. https://doi.org/10.1002/lol2.10127

Chaplin, M.F., 2001. Water: its importance to life. Biochem. Mol. Biol. Educ. 29, 54-59. https://doi.org/10.1016/S1470-8175(01)00017-0

Chen, G., Fu, Z., Yang, H., Wang, J., 2020. An overview of analytical methods for detecting microplastics in the atmosphere. Trends Anal. Chem. 115981. https://doi.org/10.1016/j.trac.2020.115981

Chokwe, T.B., Okonkwo, J.O., 2019. Occurrence, distribution and ecological risk assessment of organophosphorus flame retardants and plasticizers in sediment samples along the Vaal River catchment, South Africa. Emerg. Contam. 5, 173–178. https://doi.org/10.1016/j.emcon.2019.05.003

City Parks, 2014 [WWW Document]. A clean river runs through it. URL http://www.jhbcityparks.com/index.php/news-mainmenu-56/1110-a-clean-river-runs-through-it (accessed 7-29-19).

Collicutt, B., Juanes, F., Dudas, S.E., 2019. Microplastics in juvenile Chinook salmon and their nearshore environments on the east coast of Vancouver Island. Environ. Pollut. 244, 135–142. https://doi.org/10.1016/j.envpol.2018.09.137

Connell, L.J., van Rensburg, G.J., Avenant-Oldewage, A., Greenfield, R., 2020. Biomarker responses in African sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), as indicators of potential metal and organic pollution along the Vaal River system, South Africa. Afr J. Aquat. Sci. 1–12. https://doi.org/10.2989/16085914.2020.1735296

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829–837. https://doi.org/10.1016/j.envpol.2017.07.017

Cyrus, D.P., Wepener, V., Mackay, C.F., Cilliers, P.M., Weerst, S.P., Viljoen, A., 2000. The effects of interbasin transfer on the hydrochemistry, benthic invertebrates and ichythyofauna of the Mhlathuze Estuary and Lake Nsezi. WRC Report No. 722/1/00, Water Research Commission, Pretoria, South Africa.

Dahms, H.T.J., van Rensburg, G.J., Greenfield, R., 2020. The microplastic profile of an urban African stream. Sci. Total Environ. 731, 138893. https://doi.org/10.1016/j.scitotenv.2020.138893

Dalu, T., Malesa, B., Cuthbert, R.N., 2019. Assessing factors driving the distribution and characteristics of shoreline macroplastics in a subtropical reservoir. Sci. Total Environ. 696, 133992. https://doi.org/10.1016/j.scitotenv.2019.133992

Dickens, C., Graham, P.M., 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. Afr. J. Aquat. Sci. 27, 1-10. https://doi.org/10.2989/16085914.2002.9626569

de Sá, L.C., Oliveira, M., Ribeiro, F., Lopes, T., Futter, M., 2018. Studies of the effects of microplastics on aquatic organisms : What do we know and where should we focus our efforts in the future ? Sci. Total Environ. 645, 1029–1039. https://doi.org/10.1016/j.scitotenv.2018.07.207

de Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: Plastics debris. Mar. Pollut. Bull. 69, 206–214. https://doi.org/10.1016/j.marpolbul.2013.01.033

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018. Microplastics in mussels and fish from the Northern Ionian Sea. Mar. Pollut. Bull. 135, 30–40. https://doi.org/10.1016/j.marpolbul.2018.06.063

Dikareva, N., Simon, K.S., 2019. Microplastic pollution in streams spanning an urbanisation gradient. Environ. Pollut. 250, 292–299. https://doi.org/10.1016/j.envpol.2019.03.105

du Toit, S., 2016. [WWW Document]. Vaal Dam reaches capacity. News Rep. URL https://www.jacarandafm.com/news/news/vaal-dam-reaches-capacity/ (accessed 8-12-20).

DWAF, 2009. Department of Water Affairs. Rapid Habitat Assessment Model Manual. Report no RDM/ Nat/00/CON/0707. Authors: D Louw & CJ Kleynhans Submitted by Water for Africa.

Eerkes-Medrano, D., Thompson, R., 2018. Occurrence, Fate, and Effect of Microplastics in Freshwater Systems, Microplastic Contamination in Aquatic Environments. 95-132. https://doi.org/10.1016/B978-0-12-813747-5.00004-7

EPA., 2020. [WWW Document]. Environmental Protection Agency. Drinking water regulations and contaminants. URL https://www.epa.gov/sdwa/drinking-water-regulations-and-contaminants (accessed 8-12-20).

Feng, Z., Zhang, T., Shi, H., Gao, K., Huang, W., Xu, J., 2020. Microplastics in bloom-forming macroalgae : Distribution , characteristics and impacts. J. Hazard. Mater.

397, 122752. https://doi.org/10.1016/j.jhazmat.2020.122752

Fisher, E.M., 2011. Metal bioaccumulationand biomarker responses in tigerfish, *Hydrocynus vittatus,* from three South African populations. MSc Dissertation. University of Johannesburg. Johannesburg, South Africa.

Gatidou, G., Arvaniti, O.S., Stasinakis, A.S., 2018. Review on the occurrence and fate of microplastics in Sewage Treatment Plants. J. Hazard. Mater. 367, 504–512. https://doi.org/10.1016/j.jhazmat.2018.12.081

Geilfus, N.X., Munson, K.M., Sousa, J., Germanov, Y., Bhugaloo, S., Babb, D., Wang, F., 2019. Distribution and impacts of microplastic incorporation within sea ice. Mar. Pollut. Bull. 145, 463–473. https://doi.org/10.1016/j.marpolbul.2019.06.029

GESAMP, 2019. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Guidelines for the monitoring and assessment of plastic litter and microplastics in the ocean (Kershaw P.J., Turra A. and Galgani F. editors), (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA). Rep. Stud. GESAMP No. 99, 130p.

Giesy, J.P., Wiener, J.G., 1977. Frequency distribution of trace metal concentrations in five freshwater fishes. Trans. Am. Fish. Soc. 106, 393-403. https://doi.org/10.1577/1548-8659(1977)106<393:FDOTMC>2.0.CO;2

Gómez-Hernández, J.A., 2012. Las bibliotecas escolares en España ante una nueva década, Anuario ThinkEPI (in Spanish). https://doi.org/10.2788/99475

Google Earth, 2020. [WWW Document]. Upper Vaal River. URL https://earth.google.com/web/@26.8725028,28.04555368,1510.71139307a,105222.77068142d,35y,0h,0t,0r (accessed 8-12-20).

Greenfield, R., 2004. An assessment protocol for water quality integrity and management of the Nyl River Wetland System. PhD Thesis. University of Johannesburg. Johannesburg, South Africa.

Gregory, M.R., 1977. Plastic pellets on New Zealand beaches. Mar. Pollut. Bull. 8, 82–84. https://doi.org/10.1016/0025-326X(77)90193-X

Groenewald, A., 1964. Observations on the food habits of *Clarias gariepinus* Burchell, the South African freshwater barbel (Pisces: Clariidae) in Transvaal. Hydrobiologia. 23, 287–291. https://doi.org/10.1007/BF00043737

Guo, X., Wang, J., 2019. The chemical behaviors of microplastics in marine environment : A review. Mar. Pollut. Bull. 142, 1–14. https://doi.org/10.1016/j.marpolbul.2019.03.019

Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in marine organism: Environmental and toxicological effects. Environ. Toxicol. Pharmacol. 64, 164–171. https://doi.org/10.1016/j.etap.2018.10.009

Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics : Migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., Lei, L., 2018. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. TrAC - Trends Anal. Chem. 109, 163–172. https://doi.org/10.1016/j.trac.2018.10.006

Herrera, A., Ŝtindlová, A., Martínez, I., Rapp, J., Romero-Kutzner, V., Samper, M.D., Montoto, T., Aguiar-González, B., Packard, T., Gómez, M., 2019. Microplastic ingestion by Atlantic chub mackerel (*Scomber colias*) in the Canary Islands coast. Mar. Pollut. Bull. 139, 127–135. https://doi.org/10.1016/j.marpolbul.2018.12.022

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Envronmental Sci. Technol. 46, 3060–3075. https://doi.org/10.1021/es2031505

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Hosken, G., 2018. [WWW Document]. Sunday Times. Troops battle Vaal Sew. “invasion” Aim is to avert Day Zero Gauteng, fix collapsed sewerage. URL https://www.timeslive.co.za/sunday-times/news/2018-12-02-troops---battle-vaal--sewage-invasion/ (accessed 15-10-20).

Imhoff, H. K., Schmid, J., Niessner, R., Ivelva, N. P., Laforsch, C., 2012. A novel, highly efficient method for seperation and quantification of plastic particles in sediments

of aquatic environments. Limnol. Oceanogr. 10, 524-537. http://doi.org/10.4319/lom.2012.10.524

Iwasaki, S., Isobe, A., Uchida, K., Tokai, T., 2017. Fate of microplastics and mesoplastics carried by surface currents and wind waves : A numerical model approach in the Sea of Japan. Mar. Pollut. Bull. 121, 85–96. https://doi.org/10.1016/j.marpolbul.2017.05.057

Kane, I.A., Clare, M.A., Hodgson, D.M., Kane, I.A., 2019. Dispersion, Accumulation, and the Ultimate Fate of Microplastics in Deep-Marine Environments : A Review and Future Directions. Front. Earth. Sci. 7:80. https://doi.org/10.3389/feart.2019.00080

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P., Pohl, F., 2020. Seafloor microplastic hotspots controlle by deep-sea circulation. Science. 368, 1140–1145. https://doi.org/10.1126/science.aba5899

Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). Environ. Res. 151, 58–70. https://doi.org/10.1016/j.envres.2016.07.024

Kitahara, K.I., Nakata, H., 2020. Plastic additives as tracers of microplastic sources in Japanese road dusts. Sci. Total Environ. 736, 139694. https://doi.org/10.1016/j.scitotenv.2020.139694

Kumar, V.E., Ravikumar, G., Jeyasanta, K.I., 2018. Occurrence of microplastics in fishes from two landing sites in Tuticorin, South east coast of India. Mar. Pollut. Bull. 135, 889–894. https://doi.org/10.1016/j.marpolbul.2018.08.023

Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., Setälä, O., 2018. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. Food Webs 17, e00097. https://doi.org/10.1016/j.fooweb.2018.e00097

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

Li, J., Liu, H., Paul Chen, J., 2018. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Res. 137, 362–374. https://doi.org/10.1016/j.watres.2017.12.056

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods. 9, 1346–1360. https://doi.org/10.1039/c6ay02415g

Malherbe, W., Wepener, V., Van Vuren, J.H.J., 2016. The effect of a large-scale irrigation scheme on the fish community structure and integrity of a subtropical river system in South Africa. Ecol. Indic. 69, 533–539. https://doi.org/10.1016/j.ecolind.2016.05.005

Mani, T., Hauk, A., Wal, U., Burkhardt-holm, P., 2015. Microplastics profile along the Rhine River. Nature. 5, 1–7. https://doi.org/10.1038/srep17988

Massos, A., Turner, A., 2017. Cadmium, lead and bromine in beached microplastics. Environ. Pollut. 227, 139–145. https://doi.org/10.1016/j.envpol.2017.04.034

Mayoma, B.S., Mjumira, I.S., Efudala, A., Syberg, K., Khan, F.R., 2019. Collection of anthropogenic litter from the shores of lake Malawi: Characterization of plastic debris and the implications of public involvement in the african great lakes. Toxics. 7, 64. https://doi.org/10.3390/toxics7040064

McIlwraith, H.K., Lin, J., Erdle, L.M., Mallos, N., Diamond, M.L., Rochman, C.M., 2019. Capturing microfibers– marketed technologies reduce microfiber emissions from washing machines. Mar. Pollut. Bull. 139, 40–45. https://doi.org/10.1016/j.marpolbul.2018.12.012

MERI, 2015. Marine and Environmental Research Institute. Guide to Microplastic Identification. University of Florida. Florida, United States of America.

Moloi, M., Ogbeide, O., Voua Otomo, P., 2020. Probabilistic health risk assessment of heavy metals at wastewater discharge points within the Vaal River Basin, South Africa. Int. J. Hyg. Environ. Health 224, 113421. https://doi.org/10.1016/j.ijheh.2019.113421

Naidoo, T., Glassom, D., Smit, A.J., 2015. Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa. Mar. Pollut. Bull. 101, 473–480. https://doi.org/10.1016/j.marpolbul.2015.09.044

Näkki, P., Setälä, O., Lehtiniemi, M., 2019. Seafloor sediments as microplastic sinks in the northern Baltic Sea– Negligible upward transport of buried microplastics by bioturbation. Environ. Pollut. 249, 74–81. https://doi.org/10.1016/j.envpol.2019.02.099

Nel, H.A., Dalu, T., Wasserman, R.J., 2018. Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. Sci. Total Environ. 612, 950–956. https://doi.org/10.1016/j.scitotenv.2017.08.298

Nel, H.A., Froneman, P.W., 2015. A quantitative analysis of microplastic pollution along the south-eastern coastline of South Africa. Mar. Pollut. Bull. 101, 274–279. https://doi.org/10.1016/j.marpolbul.2015.09.043

Oberholster, P.J., Botha, A-M., Cloete, T.E., 2008. Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environ. Pol. 156, 184-192. https://doi.org/10.1016/j.envpol.2007.12.028

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175

Peixoto, D., Pinheiro, C., Amorim, J., Oliva-Teles, L., Guilhermino, L., Vieira, M.N., 2019. Microplastic pollution in commercial salt for human consumption: A review. Estuar. Coast. Shelf Sci. 219, 161–168. https://doi.org/10.1016/j.ecss.2019.02.018

Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., Du, M., Li, J., Guo, Z., Bai, S., 2018. Microplastics contaminate the deepest part of the world's ocean. Geochem. Persp. Let. 9, 1–5. https://doi.org/10.7185/geochemlet.1829

Pirsaheb, M., Hossini, H., Makhdoumi, P., 2020. Review of microplastic occurrence and toxicological effects in marine environment: Experimental evidence of inflammation. Process Saf. Environ. Prot. 142, 1–14. https://doi.org/10.1016/j.psep.2020.05.050

Plastics SA | SA Annual Review, (2017). [WWW Document]. Plastics SA | SA Annual

Review, Marketing and Communications Division. Midrand, Gauteng, South Africa. https://www.plasticsinfo.co.za/media-room/annual-reports (accessed 11-7-19).

Plessl, C., Gilbert, B.M., Sigmund, M.F., Theiner, S., Avenant-Oldewage, A., Keppler, B.K., Jirsa, F., 2018. Mercury, silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa, including implications for fish consumers. Sci. Total Environ. 659, 1158–1167. https://doi.org/10.1016/j.scitotenv.2018.12.442

Rainieri, S., Barranco, A., 2019. Microplastics, a food safety issue? Trends Food Sci. Technol. 84, 55–57. https://doi.org/10.1016/j.tifs.2018.12.009

Randwater, 2019. [WWW. Document]. Vaal Barrage and Vaal Dam daily flow information 2019. https://www.reservoir.co.za/flow.htm. (accessed 15-10-20).

Reed, S., Clark, M., Thompson, R., Hughes, K.A., 2018. Microplastics in marine sediments near Rothera Research Station, Antarctica. Mar. Pollut. Bull. 133, 460–463. https://doi.org/10.1016/j.marpolbul.2018.05.068

Retief, N., Avenant-Oldewage, A., Du Preez, H.H., 2009. Seasonal study on *Bothriocephalus* as indicator of metal pollution in yellowfish, South Africa. A. J. OL. 35, 315–322. http://doi.org/10.4314/wsa.v35i3.76769.

Rezaei, M., Riksen, M.J.P.M., Sirjani, E., Sameni, A., Geissen, V., 2019. Wind erosion as a driver for transport of light density microplastics. Sci. Total Environ. 669, 273–281. https://doi.org/10.1016/j.scitotenv.2019.02.382

Rimayi, C., Chimuka, L., Odusanya, D., de Boer, J., Weiss, J., 2016. Distribution of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofurans in the Jukskei and Klip/Vaal catchment areas in South Africa. Chemosphere. 145, 314–321. https://doi.org/10.1016/j.chemosphere.2015.11.088

Roch, S., Walter, T., Ittner, L.D., Friedrich, C., Brinker, A., 2019. A systematic study of the microplastic burden in freshwater fishes of south-western Germany- Are we searching at the right scale? Sci. Total Environ. 689, 1001–1011. https://doi.org/10.1016/j.scitotenv.2019.06.404

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D. V, Lam, R., Miller, J.T., Teh, F.,

Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood : Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci Rep. 5, 14340. https://doi.org/10.1038/srep14340

SANS. 2008. South African National Standard: The care and use of animals for scientific purposes. *Occupational Health and Safety Act*. Pretoria, South Africa: South African Bureau of Standards (SABS) Standards Division.

Silva-Cavalcanti, J.S., Silva, J.D.B., de França, E.J., de Araújo, M.C.B., Gusmão, F., 2017. Microplastics ingestion by a common tropical freshwater fishing resource. Environ. Pollut. 221, 218–226. https://doi.org/10.1016/j.envpol.2016.11.068

Skelton, P., 2001. A complete guide to the freshwater fishes of southern Africa. Cape Town, South Africa: Struik Publishers.

Slootmaekers, B., Catarci Carteny, C., Belpaire, C., Saverwyns, S., Fremout, W., Blust, R., Bervoets, L., 2019. Microplastic contamination in gudgeons (*Gobio gobio*) from Flemish rivers (Belgium). Environ. Pollut. 244, 675–684. https://doi.org/10.1016/j.envpol.2018.09.136

Stats SA, 2011. [WWW Document]. Statistics South Africa. Statistics by place. http://www.statssa.gov.za/?page_id=4286&id=11169 (assessed 15-10-20).

Stats SA, 2017. [WWW Document]. Statistics South Africa. Poverty on the Rise in South Africa. Poverty rise South Africa. URL http://www.statssa.gov.za/?p=10334 (accessed 25-07-19).

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.J., 2019. Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Res. 152, 21-37. https://doi.org/10.1016/j.watres.2018.12.050

Syakti, A.D., Jaya, J.V., Rahman, A., Hidayati, N.V., Raza'i, T.S., Idris, F., Trenggono, M., Doumenq, P., Chou, L.M., 2019. Bleaching and necrosis of staghorn coral (*Acropora formosa*) in laboratory assays: Immediate impact of LDPE microplastics. Chemosphere 228, 528–535. https://doi.org/10.1016/j.chemosphere.2019.04.156

Tunnell, J.W., Dunning, K.H., Scheef, L.P., Swanson, K.M., 2020. Measuring plastic pellet (nurdle) abundance on shorelines throughout the Gulf of Mexico using citizen scientists : Establishing a platform for policy- relevant research. Mar.

Pollut. Bull. 151, 110794. https://doi.org/10.1016/j.marpolbul.2019.110794

Turner, A., 2016. Heavy metals, metalloids and other hazardous elements in marine plastic litter. Mar. Pollut. Bull. 111, 136–142. https://doi.org/10.1016/j.marpolbul.2016.07.020

Turner, A., 2019. Hazardous elements in plastic consumer products and marine microplastics : origin and potential impacts. Sustainable Seminar Series. 12-09-19. http://hdl.handle.net/2142/106645 (accessed 12-08-20).

USEPA, 1991. United States Environmental Protection Agency. Description and Sampling of Contaminated Soils: a field pocket guide. EPA/625/12-91/002. Cincinnati, USA: United States Environmental Protection Agency.

USEPA, 2001. United States Environmental Protection Agency. Methods for collection, storage and manipulation of sediments for chemical and toxicological analyses: technical manual. EPA 823-B-01-002. United States Environmental Protection Agency, Office of Water, Washington DC, USA. Appendix G: 194–208.

Vaal Explorer, 2019. [WWW Document]. Vaal explorer. Adventures to enjoy places to visit gt. Vaal reg.– part 1. URL https://vaalexplorer.co.za/2019/09/30/adventures-to-enjoy-places-to-visit-in-the-greater-vaal-region-part-1/ (accessed 12-08-20).

Van Cauwenberghe, L. van Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008

van der Waal, B.C.W., Schoonbee, H.J., 1975. Age and growth studies of *Clarias gariepuns* (Burchell) (Clariidae) in the Transvaal, South Africa. J. Fish Biol. 7, 227–233. https://doi.org/10.1111/j.1095-8649.1975.tb04594.x

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kottermna, K.J.J., Diogene, J., Bekaert, K., Robbens, J., Devries, L., 2015. A critical view on microplastic quantification in aquatic organisms. Environ. Res. 143, 46–55. https://doi.org/10.1016/j.envres.2015.07.016

Verster, C., Minnaar, K., Bouwman, H., 2017. Marine and freshwater microplastic research in South Africa. Integr. Environ. Assess. Manag. 13, 533–535. https://doi.org/10.1002/ieam.1900

Vitule, J.R.S., Umbria, S.C., Aranha, J.M.R., 2006. Introduction of the African catfish *Clarias gariepinus* (Burchell, 1822) into Southern Brazil. Biol. Invasions. 8, 677–681. https://doi.org/10.1007/s10530-005-2535-8

Wagner, M., Scherer, C., Alvarez-muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems : What we know and what we need to know. Environ. Sci. Eur. 26, 1–9. https://doi.org/https://doi.org/10.1186/s12302-014-0012-7

Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: A review. Ecotoxicol. Environ. Saf. 173, 110–117. https://doi.org/10.1016/j.ecoenv.2019.01.113

Wang, Z.M., Wagner, J., Ghosal, S., Bedi, G., Wall, S., 2017. SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and fish guts. Sci. Total Environ. 603–604, 616–626. https://doi.org/10.1016/j.scitotenv.2017.06.047

Watkins, L., Mcgrattan, S., Sullivan, P.J., Walter, M.T., 2019. The effect of dams on river transport of microplastic pollution. Sci. Total Environ. 664, 834–840. https://doi.org/10.1016/j.scitotenv.2019.02.028

Weideman, E.A., Perold, V., Ryan, P.G., 2019. Little evidence that dams in the Orange– Vaal River system trap floating microplastics or microfibres. Mar. Pollut. Bull. 149, 110664. https://doi.org/10.1016/j.marpolbul.2019.110664

Weideman, E.A., Perold, V., Ryan, P.G., 2020. Limited long-distance transport of plastic pollution by the Orange-Vaal River system, South Africa. Sci. Total Environ. 727, 138653. https://doi.org/10.1016/j.scitotenv.2020.138653

Wepener, V., van Dyk, C., Bervoets, L., Brien, G.O., Covaci, A., Cloete, Y., 2011. An assessment of the influence of multiple stressors on the Vaal River , South Africa. Phys. Chem. Earth. 36, 949–962. https://doi.org/10.1016/j.pce.2011.07.075

Windsor, F.M., Tilley, R.M., Tyler, C.R., Ormerod, S.J., 2019. Microplastic ingestion by riverine macroinvertebrates. Sci. Total Environ. 646, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271

Xiong, X., Chen, X., Zhang, K., Mei, Z., Hao, Y., Zheng, J., Wu, C., Wang, K., Ruan, Y., Lam, P.K.S., Wang, D., 2018. Microplastics in the intestinal tracts of East Asian finless porpoises (*Neophocaena asiaeorientalis sunameri*) from Yellow Sea and Bohai Sea of China. Mar. Pollut. Bull. 136, 55–60. https://doi.org/10.1016/j.marpolbul.2018.09.006

Yao, L., Hui, L., Yang, Z., Chen, X., Xiao, A., 2020. Freshwater microplastics pollution : Detecting and visualizing emerging trends based on Citespace II. Chemosphere 245, 125627. https://doi.org/10.1016/j.chemosphere.2019.125627

Yukioka, S., Tanaka, S., Nabetani, Y., Suzuki, Y., Ushijima, T., 2020. Occurrence and characteristics of microplastics in surface road dust in Kusatsu ( Japan ), Da Nang (Vietnam), and Kathmandu (Nepal). Environ. Pollut. 256, 113447. https://doi.org/10.1016/j.envpol.2019.113447

# Annexures

# Annexure 1: Vaal Daily flow information (24-30 August 2019)

VAAL BARRAGE AND VAAL DAM DAILY FLOW INFORMATION 2019

| VAAL BARRAGE @ 07h00 | | | 24 Aug Sat | 25 Aug Sun | 26 Aug Mon | 27 Aug Tue | 28 Aug Wed | 29 Aug Thu | 30 Aug Fri |
|---|---|---|---|---|---|---|---|---|---|
| Level (m) | | | 7.55 | 7.55 | 7.56 | 7.56 | 7.54 | 7.52 | 7.47 |
| Flow ($m^3/s$) | | | 10.127 | 10.127 | 10.134 | 10.134 | 10.120 | 10.107 | 10.073 |
| Average flow day before ($m^3/s$) | | | 10.127 | 10.127 | 10.130 | 10.134 | 10.122 | 10.109 | 10.081 |
| Minimum flow day before ($m^3/s$) | | | 10.127 | 10.127 | 10.127 | 10.134 | 10.120 | 10.107 | 10.073 |
| Maximum flow day before ($m^3/s$) | | | 10.127 | 10.127 | 10.134 | 10.134 | 10.134 | 10.120 | 10.107 |
| Rainfall day before (mm) | | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Evaporation (mm) | | | 3.0 | 4.0 | 3.0 | 2.5 | 4.5 | 2.0 | 4.0 |
| Conductivity (mS/ml) | | | 77.8 | 78.0 | 75.8 | 82.2 | 78.5 | 77.5 | 80.2 |
| Lethabo river level (m) | | | 2.52 | 2.53 | 2.52 | 2.50 | 2.51 | 2.49 | 2.50 |
| **No. of gates open** | **Inches open** | **Flow / gate ($m^3/s$)** | **Temp (°C)** | **Date** | **Notes** | | | | |
| 1 | 1X6" | 10.127 | 13.0 | 24 Aug | | | | | |
| 1 | 1X6" | 10.127 | 12.6 | 25 Aug | | | | | |
| 1 | 1X6" | 10.134 | 11.6 | 26 Aug | | | | | |
| 1 | 1X6" | 10.134 | 13.4 | 27 Aug | | | | | |
| 1 | 1X6" | 10.120 | 13.1 | 28 Aug | | | | | |
| 1 | 1X6" | 10.107 | 12.9 | 29 Aug | | | | | |
| 1 | 1X6" | 10.073 | 13.9 | 30 Aug | | | | | |

| VAAL DAM @ 06h00 | 24 Aug Sat | 25 Aug Sun | 26 Aug Mon | 27 Aug Tue | 28 Aug Wed | 29 Aug Thu | 30 Aug Fri |
|---|---|---|---|---|---|---|---|
| Level (m) | 18.900 | 18.890 | 18.860 | 18.850 | 18.830 | 18.800 | 18.770 |
| Full (%) | 61.12 | 61.03 | 60.76 | 60.67 | 60.49 | 60.22 | 59.95 |
| Inflow ($m^3/s$) | 7.83 | 7.39 | 19.27 | 19.28 | 9.68 | 9.68 | 13.98 |
| Discharge ($m^3/s$) | 18.03 | 18.03 | 18.03 | 17.19 | 17.19 | 17.19 | 17.19 |
| Number of river valves open and % discharge | 2x100% | 2x100% | 2x100% | 2x100% | 2x100% | 2x100% | 2x100% |
| Number of gates open | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Information supplied on behalf of Rand Water and the Department Water & Sanitation
Please Note: Not all abstraction and evaporation losses from Vaal Dam are reflected in the above data

(Randwater, 2020)

# Annexure 2: Vaal daily flow information (31 Aug-6 September)

VAAL BARRAGE AND VAAL DAM DAILY FLOW INFORMATION 2019

| VAAL BARRAGE @ 07h00 | | | 31 Aug Sat | 1 Sep Sun | 2 Sep Mon | 3 Sep Tue | 4 Sep Wed | 5 Sep Thu | 6 Sep Fri |
|---|---|---|---|---|---|---|---|---|---|
| Level (m) | | | 7.47 | 7.45 | 7.44 | 7.43 | 7.41 | 7.41 | 7.42 |
| Flow ($m^3/s$) | | | 10.073 | 10.059 | 10.052 | 10.046 | 10.031 | 10.031 | 10.039 |
| Average flow day before ($m^3/s$) | | | 10.073 | 10.061 | 10.058 | 10.045 | 10.034 | 10.031 | 10.035 |
| Minimum flow day before ($m^3/s$) | | | 10.073 | 10.059 | 10.052 | 10.046 | 10.031 | 10.031 | 10.031 |
| Maximum flow day before ($m^3/s$) | | | 10.073 | 10.073 | 10.059 | 10.052 | 10.046 | 10.031 | 10.039 |
| Rainfall day before (mm) | | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Evaporation (mm) | | | 4.0 | 4.0 | 3.0 | 4.0 | 3.0 | 2.0 | 3.5 |
| Conductivity (mS/ml) | | | 79.8 | 78.2 | 77.3 | 75.6 | 79.0 | 79.0 | 81.9 |
| Lethabo river level (m) | | | 2.52 | 2.52 | 2.52 | 2.50 | 2.50 | 2.51 | 2.51 |
| **No. of gates open** | **Inches open** | **Flow / gate ($m^3/s$)** | **Temp (°C)** | **Date** | **Notes** | | | | |
| 1 | 1X6" | 10.073 | 13.6 | 31 Aug | | | | | |
| 1 | 1X6" | 10.059 | 12.2 | 1 Sep | | | | | |
| 1 | 1X6" | 10.052 | 12.6 | 2 Sep | | | | | |
| 1 | 1X6" | 10.046 | 11.3 | 3 Sep | | | | | |
| 1 | 1X6" | 10.031 | 12.8 | 4 Sep | | | | | |
| 1 | 1X6" | 10.031 | 13.0 | 5 Sep | | | | | |
| 1 | 1X6" | 10.039 | 14.5 | 6 Sep | | | | | |

| VAAL DAM @ 06h00 | 31 Aug Sat | 1 Sep Sun | 2 Sep Mon | 3 Sep Tue | 4 Sep Wed | 5 Sep Thu | 6 Sep Fri |
|---|---|---|---|---|---|---|---|
| Level (m) | 18.750 | 18.730 | 18.700 | 18.670 | 18.650 | 18.630 | 18.510 |
| Full (%) | 59.77 | 59.60 | 59.33 | 59.06 | 58.89 | 58.71 | 58.54 |
| Inflow ($m^3/s$) | 12.77 | 11.32 | 10.92 | 10.35 | 11.20 | 21.98 | 23.53 |
| Discharge ($m^3/s$) | 17.19 | 17.19 | 17.19 | 17.19 | 17.19 | 17.19 | 17.19 |
| Number of river valves open and % discharge | 2x100% | 2x100% | 2x100% | 2x100% | 2x100% | 2x100% | 2x100% |
| Number of gates open | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Information supplied on behalf of Rand Water and the Department Water & Sanitation
Please Note: Not all abstraction and evaporation losses from Vaal Dam are reflected in the above data

(Randwater, 2020)

# Annexure 3: Author statement Chapter 3

## Chapter 3 Author statement

**(Dahms et al., 2020)**

Dahms, H.T.J., van Rensburg, G.J., Greenfield, R., 2020. The microplastic profile of an urban African stream. Sci. tot. Env. 731. 138893. https://doi.org/10.1016/j.scitotenv.2020.138893

### CRediT authorship contribution statement

**Heinrich T.J. Dahms:** Writing - original draft, Formal analysis, Visualization, Investigation. **Gregg J. van Rensburg:** Formal analysis, Writing - review & editing, Visualization, Conceptualization. **Richard Greenfield:** Supervision, Writing - review & editing, Funding acquisition.

# Annexure 4: First page of published chapter

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## The microplastic profile of an urban African stream

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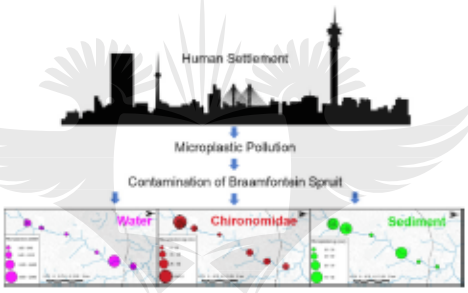
### HIGHLIGHTS

- Microplastics had a 100% prevalence in Chironomid larvae groups.
- Stream characteristics could influence microplastic distribution.
- Microplastic abundances varied depending on what matrix was being analysed.
- A weir decreased microplastic loads downstream in sediment and invertebrates.

### GRAPHICAL ABSTRACT

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### ABSTRACT

Microplastics are small plastic fragments that have been found around the world, however, research into microplastics in Africa and freshwater systems remains insufficient. In this study, the snapshot microplastic profile of an urban stream was assessed in the Braamfontein Spruit, located in Johannesburg the largest city in South Africa. The abundance of microplastics was determined in water, *Chironomus* sp. larvae and sediment, while *in situ* parameters were taken to investigate their relationship to the microplastic profile of the different matrices. Microplastics were detected in water (mean of 705 particles $m^{-3}$), *Chironomus* sp. larvae (mean of 53.4 particles $g^{-1}$ wet weight) and sediment (mean of 166.8 particles $kg^{-1}$ dry weight). The study found evidence of how urban stream characteristics such as a weir, stream depth and velocity could affect the abundance and dispersion of microplastics. The results indicate that areas of increased depth and decreased flow allowed microplastics to settle down to the sediment where benthic macroinvertebrates could ingest these fragments. Large obstructions like a weir also increased microplastic counts in sediment and invertebrates above the obstruction, with a decrease of fragments after the obstruction, however, microplastics in surface water were able to flow over the obstruction and increase in abundance downstream. This study concludes that first order urban streams such as the Braamfontein Spruit may be contributing large numbers of microplastics to higher order streams and large rivers in times of increased flow.

(Dahms et al., 2020)

# Annexure 5: Supplementary data

**Table 3 Results of Kolmogorov-Smirnov and Shapiro Wilk normality test on plastic/fish units used in the upper Vaal River, Chapter 4.**

| | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Particles $g^{-1}$ gut ww | .201 | 39 | .000* | .762 | 39 | .000* |
| Particles $g^{-1}$ fish | .178 | 39 | .003* | .831 | 39 | .000* |
| Particles per fish | .213 | 39 | .000* | .841 | 39 | .000* |

a. Lilliefors Significance Correction

**Table 4: Results of Spearman's rank correlation on plastics/fish units used in Chapter 4.**

| Significant*=(p<0.01) | | | Particles $g^{-1}$ gut ww | Particles $g^{-1}$ fish | Particles per fish |
|---|---|---|---|---|---|
| Spearman's rho | Particles $g^{-1}$ gut ww | Correlation | 1.000 | .962 | .828 |
| | | Sig. (2-tailed) | . | .000* | .000* |
| | | N | 39 | 39 | 39 |
| | Particles $g^{-1}$ fish | Correlation | .962 | 1.000 | .870 |
| | | Sig. (2-tailed) | .000* | . | .000* |
| | | N | 39 | 39 | 39 |
| | Particles per fish | Correlation | .828 | .870 | 1.000 |
| | | Sig. (2-tailed) | .000* | .000* | . |
| | | N | 39 | 39 | 39 |

**Table 5: Results of Kolmogorov-Smirnov and Shapiro Wilk normality test on distribution of plastics in water, fish and sediment in the upper Vaal River.**

| | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| Water | .277 | 4 | . | .907 | 4 | .464 |
| Fish | .241 | 4 | . | .898 | 4 | .421 |
| Sediment | .340 | 4 | . | .846 | 4 | .214 |

a. Lilliefors Significance Correction

**Table 6: Test of Homogeneity of Variance between microplastic levels in the matrices (Water, fish, sediment), tested in the upper Vaal River.**

| | | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|---|
| Matrices | Based on Mean | 1.986 | 2 | 9 | .193 |
| | Based on Median | 1.868 | 2 | 9 | .210 |
| | Based on Median and with adjusted df | 1.868 | 2 | 8.149 | .215 |
| | Based on trimmed mean | 2.010 | 2 | 9 | .190 |

**Table 7: Results of Pearson's correlation on the distribution of microplastics in water, fish and sediment in the upper Vaal River**

| | | Water | Fish | Sediment |
|---|---|---|---|---|
| Water | Pearson Correlation | 1 | -.412 | -.883 |
| | Sig. (2-tailed) | | .588 | .117 |
| | N | 4 | 4 | 4 |
| Fish | Pearson Correlation | -.412 | 1 | .428 |
| | Sig. (2-tailed) | .588 | | .572 |
| | N | 4 | 4 | 4 |
| Sediment | Pearson Correlation | -.883 | .428 | 1 |
| | Sig. (2-tailed) | .117 | .572 | |
| | N | 4 | 4 | 4 |

**Table 8: Results of Kolmogorov-Smirnov and Shapiro Wilk normality test on distribution of microplastics samples collected in water of the various sites of the upper Vaal River.**

| | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| **ADW** | **.361** | **6** | **.014*** | **.722** | **6** | **.011*** |
| VD | .140 | 6 | .200* | .993 | 6 | .996 |
| LVC | .230 | 6 | .200* | .883 | 6 | .283 |
| VR | .207 | 6 | .200* | .939 | 6 | .650 |

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Table 9: Table 9: Results of Mann-Whitney U test between water microplastic levels in VD and ADW of the upper Vaal River.**

| Significant*=(p<0.01) | H20 |
|---|---|
| Mann-Whitney U | .000 |
| Wilcoxon W | 21.000 |
| Z | -2.882 |
| Asymp. Sig. (2-tailed) | .004* |
| Exact Sig. [2*(1-tailed Sig.)] | .002*[b] |

a. Grouping Variable: Sites

b. Not corrected for ties.

**Table 10: Results of Mann-Whitney U test between water microplastic levels in LVC and ADW of the upper Vaal River.**

| Significant*=(p<0.01) | MP.H20 |
|---|---|
| Mann-Whitney U | .500 |
| Wilcoxon W | 21.500 |
| Z | -2.807 |
| Asymp. Sig. (2-tailed) | .005* |
| Exact Sig. [2*(1-tailed Sig.)] | .002*[b] |

a. Grouping Variable: Sites

b. Not corrected for ties.

**Table 11: Results of Mann-Whitney U test between water microplastic levels in VR and ADW of the upper Vaal River.**

| Significant*=(p<0.01) | MP.H20 |
|---|---|
| Mann-Whitney U | .500 |
| Wilcoxon W | 21.500 |
| Z | -2.807 |
| Asymp. Sig. (2-tailed) | .005* |
| Exact Sig. [2*(1-tailed Sig.)] | .002*[b] |

a. Grouping Variable: Sites

b. Not corrected for ties.